FRACTIONAL CO_2, LONG PULSE Nd:YAG AND PULSED DYE LASER IN THE MANAGEMENT OF KELOIDS

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In the fulfilment of the regulations for the award of the degree

M.D.

DERMATOLOGY, VENEREOLOGY AND LEPROLOGY

DEPARTMENT OF DERMATOLOGY, VENEROLOGY AND LEPROLOGY

PSG INSTITUTE OF MEDICAL SCIENCE AND RESEARCH

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

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GUIDE

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CERTIFICATE

This is to certify that the thesis entitled **"FRACTIONAL CO_2, LONG PULSE Nd:YAG AND PULSED DYE LASER IN THE MANAGEMENT OF KELOIDS"** is a bonafide work of **Dr. ASHWINI ANNABATHULA** done under the direct guidance and supervision of **Dr. C.R. SRINIVAS, MD** and **Dr. SHANMUGA SEKAR, MD,** in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of Dr. MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

Dr. C.R.SRINIVAS
Professor & Head of Dept.
Dept. of DVL

Dr. SHANMUGA SEKAR C.
Professor,
Dept. of DVL

Dr. RAMALINGAM
DEAN

DECLARATION

I hereby declare that this dissertation entitled **"FRACTIONAL CO_2, LONG PULSE Nd:YAG AND PULSED DYE LASER IN THE MANAGEMENT OF KELOIDS"** was prepared by me under the direct guidance and supervision of **Dr.C.R.SRINIVAS, MD** and **Dr. SHANMUGA SEKAR C., MD,** PSG Institute of Medical Sciences and Research, Coimbatore.

The dissertation is submitted to the Tamil Nadu Dr. MGR Medical University in fulfillment of the University regulation for the award of MD degree in Dermatology, Venereology and Leprology. This dissertation has not been submitted for the award of any other Degree or Diploma.

Dr. ASHWINI ANNABATHULA

CERTIFICATE BY THE GUIDE

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Dr. C. R. SRINIVAS
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Institutional Human Ethics Committee

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To
Dr Ashwini Annabathula
Postgraduate
Department of Dermatology
PSG IMS & R
Coimbatore

Ref: Project No. 14/414

Date: April 9, 2015

Dear Dr Ashwini Annabathula,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 09.01.2015 to conduct the research study entitled "Fractional CO2, Long pulse ND:YAG and pulsed dye laser in the management of keloids" during the IHEC review held on 24.02.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol
3. Informed consent form
4. Data collection tool
5. Current CVs of Principal investigator, Co-investigators
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 24.02.2015 at College Council Room, PSG IMS & R between 2.00 pm and 4.30 pm:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mrs Y Ashraf	MPT	Physiotherapy	Female	Yes	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mr P Karuppuchamy	M Phil in PSW	Social Scientist	Male	Yes	Yes
5	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	Yes
6	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
7	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	No

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8	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	No
9	Mrs P Rama	M Pharm	Non-Medical (Pharmacy)	Female	Yes	Yes
10	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Ophthalmology)	Male	No	No
11	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	Yes
12	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	No
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	No
14	Mrs. Swasthika Soundararaj	MBA	Lay person	Female	No	Yes
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC

and only then can they be implemented

f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review

7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

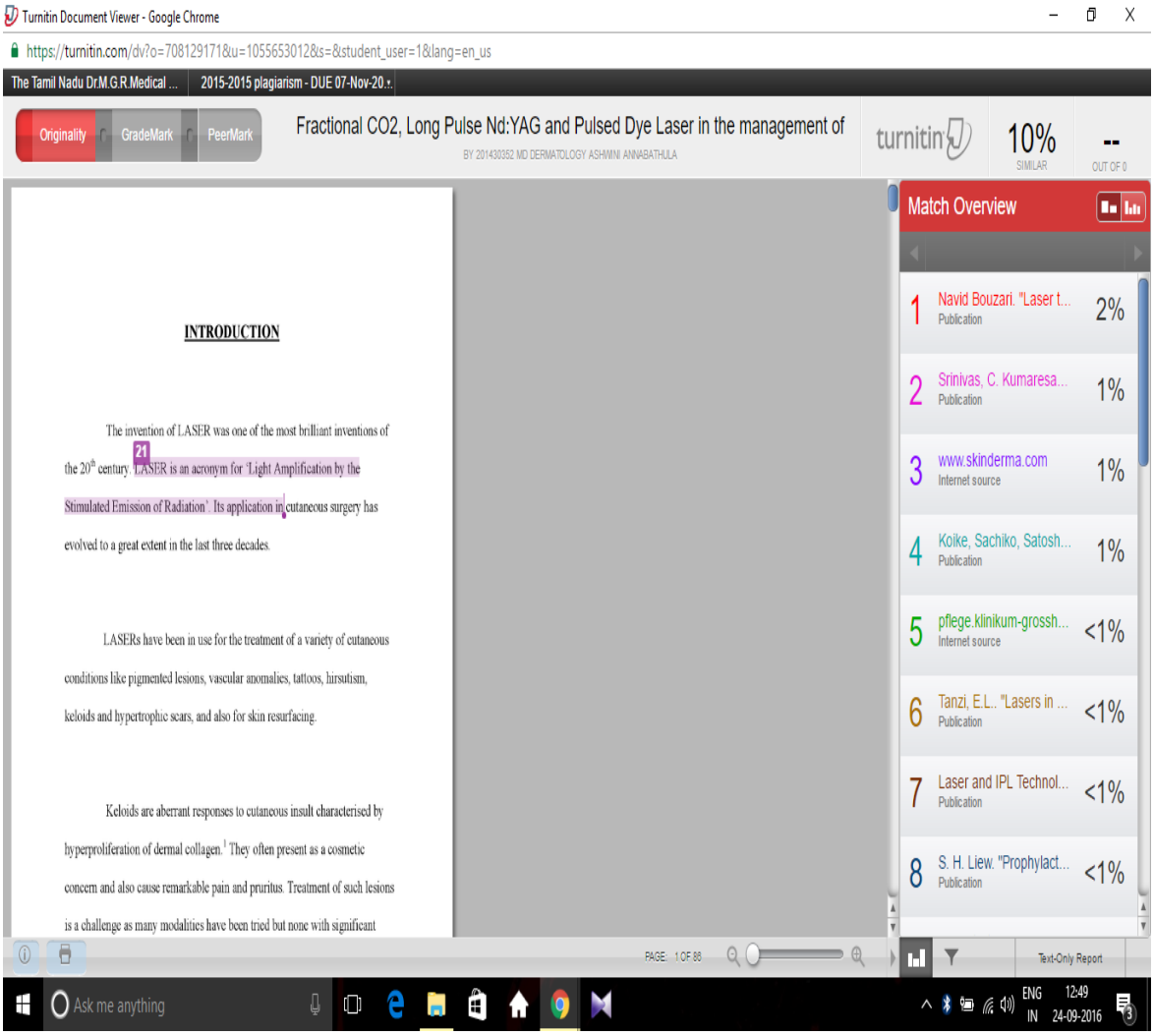
Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member-Secretary
Institutional Human Ethics Committee

PLAGIARISM TOOL



ACKNOWLEDGEMENT

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TABLE OF CONTENTS

INTRODUCTION

The invention of LASER was one of the most brilliant inventions of the 20th century. LASER is an acronym for 'Light Amplification by the Stimulated Emission of Radiation'. Its application in cutaneous surgery has evolved to a great extent in the last three decades.

LASERs have been in use for the treatment of a variety of cutaneous conditions like pigmented lesions, vascular anomalies, tattoos, hirsutism, keloids and hypertrophic scars, and also for skin resurfacing.

Keloids are aberrant responses to cutaneous insult characterised by hyperproliferation of dermal collagen.[1] They often present as a cosmetic concern and also cause remarkable pain and pruritus. Treatment of such lesions is a challenge as many modalities have been tried but none with significant improvement. Conventional methods for the treatment of keloids include surgical excision, dermabrasion, grafting, radiation, pressure therapy, silicone gel sheeting and intralesional corticosteroids.[2,3]

LASERs have been used with variable success in the treatment of keloids. Nd:YAG (Neodymium-doped yttrium aluminium garnet) and CO_2 lasers were earlier used with promising results but recent reports have not proven their long term efficacy.[4]

However, in the last 20 years, 585-nm pulsed dye laser has been very effectively used to reduce the scar erythema, size and associated symptoms.

AIMS AND OBJECTIVES

To assess the efficacy of fractional CO_2, long pulse Nd:YAG and pulsed dye LASER in the management of keloids.

REVIEW OF LITERATURE

LASERs have gained considerable attention from the dermatologic and plastic surgery communities in recent years. The term 'LASER' was coined in 1957 by Gordon Gould who is known as the 'pioneer of lasers'.[5] It is an acronym for 'Light Amplification by Stimulated Emission of Radiation'.

Richard Gordon Gould

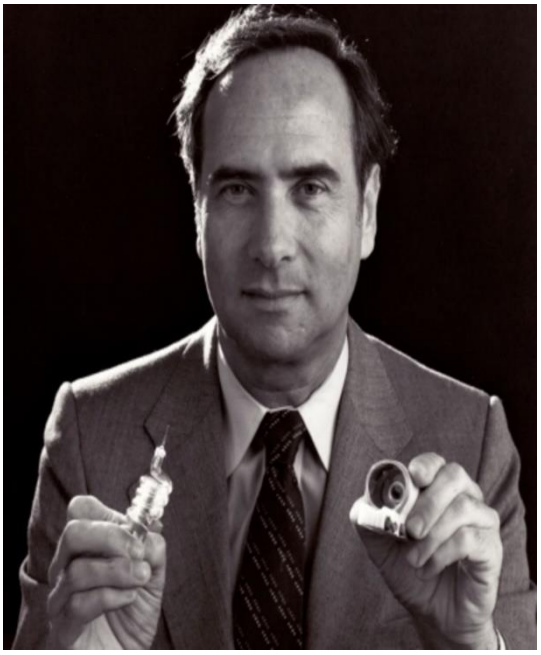
17 July, 1920 – 16 September 2005

History of Lasers

The phenomenon of stimulated light emission was put forward by Einstein in 1917. He proposed that a photon of electromagnetic energy could stimulate the emission of another identical photon from atoms or molecules that are in an excited state.[6] Einstein's magnanimous work on

laser was published in an article titled‘Zur Quantentheorie der Strahlung’ which means the ‘Quantum Theory of Radiation’.

Theodore Maiman, a physicist from California developed the first laser in 1959 using a ruby crystal to produce red light with a wavelength of 694nm.[7]Dr Leon Goldman pioneered the utility of lasers for dermatological applications by promoting ruby laser therapy for avariety of cutaneous pathologies in 1963.[8-10]



Theodore Harold Maiman

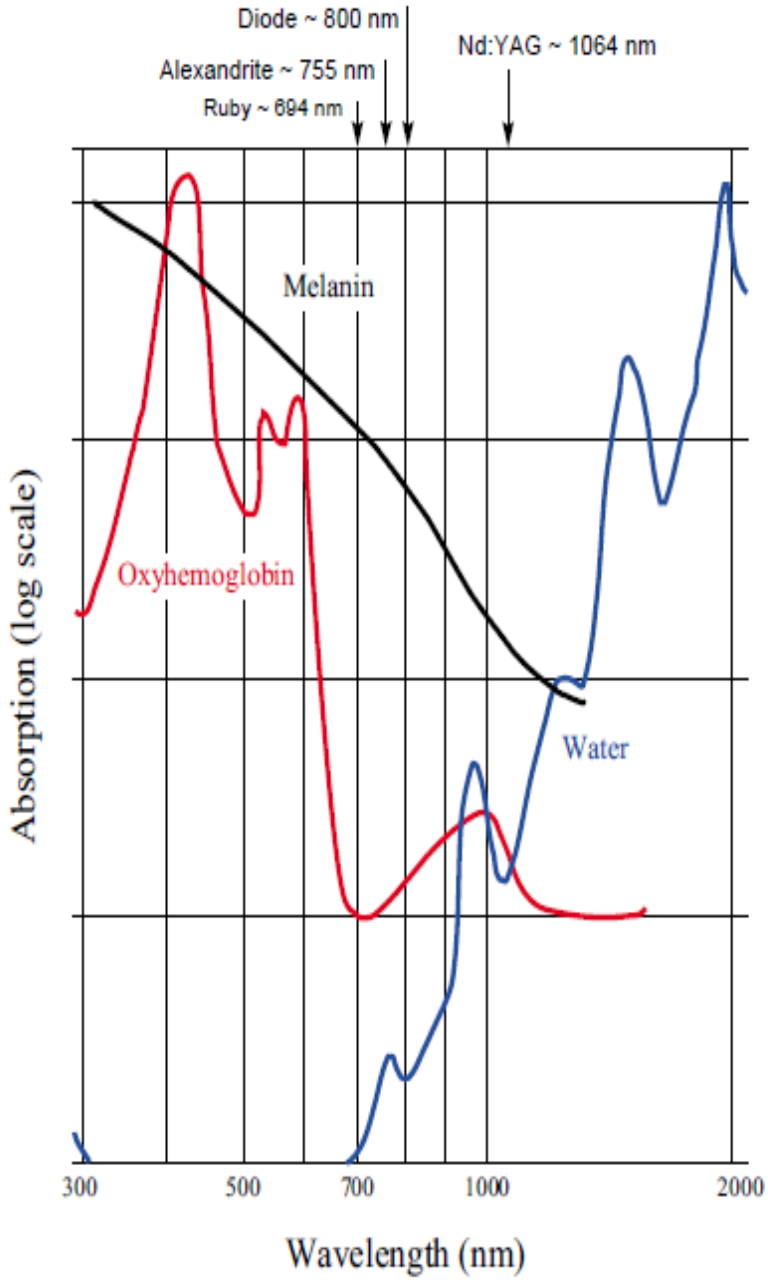
11 July, 1927 – 5 May, 2007

The Argon laser which emitted blue-green light with a wavelength of 488/514 nm, was developed in 1962 and was mainly used for the treatment of vascular lesions. Later, in 1964, the carbondioxide (CO_2) laser which produced infrared light with a wavelength of 10,600 nm was developed. This was primarily used for tissue vapourization and destruction of a variety of cutaneous lesions.[11]

There was a revolution in the field of cutaneous laser surgery in the early 80s when the theory of '**selective photothermolysis**' was proposed by Anderson and Parrish.[12]

The application of this concept of 'selective photothermolysis' allowed specific and controlled destruction of a target with a very little damage to the surrounding normal tissue. To achieve this, a specific wavelength that is absorbed by the target is selected and the duration of exposure of the tissue, i.e. the **pulse duration** must be kept shorter than 'the time taken by the targeted tissue to dissipate half the absorbed heat', which is known as its '**thermal relaxation time**'.

These targets that absorb laser of a particular wavelength are known as '**chromophores**' and they do so when their absorption spectrum matches with the wavelength of that particular laser. Some of the common 'chromophores' in the human skin are haemoglobin, melanin and water.



The absorption of different chromophores and the wavelengths of LASERs[13]

Principles of Lasers

LASER light has certain unique properties due to which it has the desired therapeutic effects.

1. **Monochromatic**, which means that the emitted light has a distinct, single wavelength.
2. **Coherence,** which means that the laser travels in phase with peaks and troughs of the light all aligned.
3. **Collimation,** which indicates non-divergent, parallel propagation of a narrow and intense beam of light.

According to the 'Grotthus-Draper law', which is the primary 'law of photobiology', light has to be absorbed by the tissue, in order to cause a clinical effect. And, the reflected, transmitted or scattered light does not affect the tissue.

After the absorption of laser by the target, 3 possible effects can occur:

1. Photothermal effect: This occurs when a chromophore absorbs energy of the corresponding wavelength and gets destroyed as a result of conversion of the absorbed energy into heat.
2. Photochemical effect: Photo-sensitizer – like reactions

3. Photomechanical effect: This occurs as a result of acoustic waves due to extreme thermal expansion.

Laser Parameters

- Laser energy is measured in joules (J)
- The amount of energy delivered per unit area (J/cm^2) is called **Fluence**. It is also known as energy density.
- **Power**(measured in watts)is the rate at which energy is delivered.

  (1 watt = 1 J/sec)
- The **wavelength** is determined by the lasing 'medium' of each laser. It is the active medium contained in an optical cavity through which the laser light passes.
- It is either a gas (argon or CO_2), liquid (dye like rhodamine, fluorescein, malachite green etc.), or a solid (ruby, alexandrite, neodymium : yttrium-aluminium-garnet crystal)

Modes of Operation

- **Continuous wave:** Emission of a constant beam of light. The exposure durations are long and may lead to non-selective tissue injury. Ex: Continuous wave CO_2 laser, Argon lasers (old).

- **Quasi-Continuous wave**: Shuttering of the continuous wave of beam into short segments, thus causing interrupted emission of constant energy. Ex: KTP, copper bromide, argon-pumped tunable dye(APTD), krypton lasers etc.

- **Pulsed wave:** Emission of high energy laser with ultrashort pulse durations. These may be long-pulsed, ex: PDL, or short-pulsed, ex: Q-switched ruby, alexandrite or Nd:YAG lasers.

  Superpulsed: Specifically used for carbondioxide lasers that produce very short pulses at a particular repetition, thereby, reducing the thermal damage of the surrounding tissue.

- **'Quality-switched' (Q-switched)** – emit high energy pulses with very short pulse durations.

LASERs in Dermatology

There has been a magnificient advancement in the field of lasers in dermatology over the last three decades. It is quite well-known that this revolution has changed the face of aesthetics and dermato-medicine.

Lasers have been in use for a variety of skin conditions like pigmented lesions, vascular anomalies, tattoos, hirsutism, keloids and hypertrophic scars, and also for skin resurfacing.

Basic lasers used in dermatology

Type of LASER	Wavelength	Chromophore	Use
Pulsed dye	585 nm / 595 nm	Oxyhaemoglobin	Vascular lesions, Keloids
Nd:YAG (freq. doubled)	532 nm	Pigment	Superficial pigment, tattoos (red, orange, yellow)
Q-switched Ruby	694 nm	Pigment	Tattoos (blue, black, green)
Ruby (Long pulsed)	694 nm	Melanin	Epilation
Alexandrite (Q-switched)	755 nm	Pigment	Tattoos (blue, black, green)
Long pulsed alexandrite	755 nm	Melanin	Epilation
Q-switched Nd:YAG	1064 nm	Pigment	Tattoos (black, blue)
Long pulsed Nd:YAG	1064 nm	Melanin	Epilation
Erbium:YAG	2940 nm	Water	Superficial skin resurfacing, epidermal lesions
Carbondioxide	10,600 nm	Water	Skin resurfacing, epidermal and dermal lesions

KELOIDS

Keloids and hypertrophic scars occur as a result of abundant deposition of collagen, the cause of which is unclear. They give rise to both functional and aesthetic distress to the patient and have always been a therapeutic challenge, despite the innumerable modalities of treatment. It is very important to define the most appropriate therapy which is not possible without a clear knowledge of the pathogenesis and the clinical characteristics of the scar.

'Keloid' was derived from the greek word 'chele' which means crab claw by Alibert, in order to demonstrate its claw-like extension into the surrounding tissue.[14]

Keloidal scars may cause symptoms like pain, pruritus, restriction of movements and also aesthetic disfigurement leading to a significant amount of morbidity.

However, keloids differ from hypertrophic scars clinically in many aspects. Hypertrophic scars are confined to the original wound and develop within weeks after injury, unlike keloids, which occur months to years after

the skin insult and surpass its boundaries. Keloids do not tend to regress but hypertrophic scars show remission as the time progresses.[15,16] Keloids have greater chances of recurrence and are more recalcitrant to treatment when compared to hypertrophic scars.[17]

Keloids are seen in predisposed individuals after injuries including surgery, burns, tattooing, piercing, abrasions, lacerations and inflammatory conditions like folliculitis, acne, chicken pox etc.[18,19] Very rarely, they show a spontaneous development.[20]

They are more common in individuals with darker skin but are found with similar frequency in both males and females.[21,22] They may start at any age, however, are more common between the 2nd and 4th decades of life.,[16,23]

Clinically, they are soft or firm or doughy in texture and may vary in diameter from a few millimetres to several centimetres. They are predominantly seen over the chest, upper back and arms, shoulders, face, ear lobes etc., cause of which could be the high skin tension of these areas.[19]

Along with the aesthetic disfigurement that they cause, they often also result in pruritus, pain, super added infection, ulceration, tightening thereby, leading to restriction of movement.[24,25]

Wound Healing

Normal wound repair is a critical process that involves the balanced and meticulously timed activity of various inflammatory, connective tissue, vascular and epithelial cells. It consists of overlapping phases of hemostasis, inflammation, proliferation and remodelling.

On injury of skin, there is a release of chemotactic and vasoactive factors that lead to the migration of inflammatory cells. Several cytokines that play a crucial role in healing by modifying the wound environment are released by the macrophages. In the next phase, fibroblasts aggregate in the site of injury and produce a structural framework by the deposition of type I and type III collagen.

Normally, in the stage of maturation, there is a decreased hyperemia and angiogenesis along with concurrent synthesis and degradation of collagen resulting in diminished nodularity and thereby, scar flattening.[2]

Pathophysiology of Keloid formation

Although the proper pathophysiologic mechanism in the formation of hypertrophic scars and keloids is not clear, many authors have published their meticulous work on the microcellular mechanisms involving the development of scars. It has been investigated that the activity of fibroblasts, various growth factors, components of extracellular matrix, cytokines and immune response portray the cellular basis of exaggerated fibrogenesis leading to the formation of a keloid.

The keloid has fibroblasts that differ in properties from those present in the normal skin. Most importantly, they have an aberrant response to injury and a higher ability of proliferation, producing excessive amount of collagen (majorly type I collagen), proteoglycans, elastin and fibronectin.[26-32]

On the other hand the fibroblasts of hypertrophic scars show a normal response to the growth factors and delineate a normal increase in the production of collagen.[33]

Growth factors that play a pivotal role in the wound healing cascade include:

1. TGF β – Transforming growth factor – β
2. Platelet derived growth factor
3. Insulin – like growth factor (IGF)

Function of TGF–β

The platelets release the transforming growth factor – β that urges the macrophages and monocytes to produce extracellular matrix proteins. It has been proved by many authors that there is a precise association between the increased synthesis of collagen by the fibroblasts of keloids and the TGF-β.[28,33,34-36]

Function of IGF

Expression of type 1 and type 3 procollagen is exponentially increased by the insulin-like growth factor-1[37] and its receptor (IGF-1 receptor) is abundantly seen in the fibroblasts of keloids.[38]

As proposed by several scientists, erratic levels of different cytokines like interleukin-6, 13 and 15 have a crucial role in the development of keloids.[38-40]

Hyaluronic acid, which is an extracellular matrix protein, binds to the receptors located on the surface of fibroblasts, and is said to cause localisation of TGF-β to the fibroblasts, thereby stimulating the synthesis of collagen.

However, there has always been a conflict of opinion in the levels of hyaluronic acid, as few researchers found increased synthesis of hyaluronic acid in the fibroblasts of keloids in comparison with those of the normal skin, while some have demonstrated decreased production in the keloidal dermis.[42,43]

A proportion of authors proposed a relationship with the expression of HLA class II molecules,[44,45] while some demonstrated elevated levels of immunoglobulins.[46]

Other pathogenic hypotheses include:

1. Hypoxia of the tissue[47]
2. Changes in the composition of fatty acids of fibroblasts[48]
3. Increased production of nitric acid in the wound repair[49]
4. Possibility of immune response to sebum[50]

A keloid on the chest

Histopathology of Keloid

It is very difficult to differentiate a keloid from hypertrophic scar histopathologically. Normal skin shows distinguishable collagen bundles running parallel to the epidermis.

They are flattened, less distinct and are arranged in a wavy pattern with a parallel orientation to the epithelium in hypertrophic scars.

However, in a keloid, the bundles of collagen are non-existent virtually and the fibres are arranged loosely in indistinctly oriented sheets.

Hypertrophic scars show nodular structures containing collagen and fibroblasts, which are however, absent in normal skin and most of the keloids.

Treatment of Keloids

As it is a well-known fact that the treatment of keloids has been a therapeutic challenge, despite good understanding of the wound repair mechanism and expertise in the field of medicine, it is of utmost importance to remember that prevention of keloids is the best possible treatment available.

Therefore, it is essential to counsel patients who are predisposed to develop thick scarring to be careful not to injure themselves and to avoid unnecessary surgeries or body piercings.

However, if a keloid develops, there are many ways of therapy to choose from, depending on the requirements of the patient, while keeping the socio-economic status in mind, although, there is no globally accepted treatment modality that can permanently cause regression of a keloid.

Various treatment options

a) Surgery
b) Radiation
c) Pressure/Compression therapy or garments
d) Cryotherapy
e) Silicon get sheets
f) Intralesional corticosteroids
g) Interferon alpha
h) 5-Fluorouracil
i) Intralesional bleomycin
j) LASER
    - Carbondioxide
    - Erbium:YAG / Nd:YAG
    - Pulsed dye laser

Surgery

All the surgically excised keloids usually recur as the newly created wound is affected by similar biological and mechanical forces of the original scar.

It has been reported that the recurrence rate may range upto 100%, if excision is chosen as an individual modality (or monotherapy).[21,51,52]

It is also believed that the keloids that have reappeared post-surgery tend to recur if surgical excision is performed again.[18,53]

Another procedure called ‘core extirpation’ is the excision of the fibrous core keeping the capsule intact, in continuation with the normal skin which acts as a flap.

Radiation

It is usually used in combination with surgical excision and not very frequently used as monotherapy. A dose of approximately 1500 Gy are delivered (in fractions) within 10 days after surgery. It acts by inhibition of angiogenesis and inhibition of the proliferation of fibroblasts during the process of wound repair.

Pressure/Compression therapy

This modality of treatment was first performed in the 60s. It is hypothesised that the continuous delivery of pressure results in tissue ischemia, thereby hindering the metabolism of the tissue and increasing the activity of the collagenase.[54,55,56]

There is another theory which states that the release of metalloproteinase / PG E2 due to the pressure therapy causes remodelling of the extracellular matrix, thereby softening the keloid.

Despite being proved to be effective in several studies, there are many demerits of pressure therapy. The most uncomfortable part is the necessity to wear it for more than 24 weeks for about 18 hours a day. Older scars do not respond well and also bringing about the required pressure of about 24-40 mm Hg is quite challenging in areas of joints where the skin is mobile.

This always results in very low patient compliance as it is uncomfortable and irritating.

Cryotherapy

It is employed either individually or in combination with other modalities of treatment for the therapy of keloids and hypertrophic scars. The mechanism by which it causes the regression of the scar is reported to be due to the freezing-induced ischemia of tissue caused by damage of the microvasculature.

Freezing

Vascular damage

Stasis of microcirculation

Hypoxia

Necrosis

Each keloid is treated with 2-3 freeze-thaw cycles, each of 30 seconds.

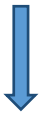
It is not very desirable in patients with darker shades of skin because it may result in permanent hypopigmentation as the melanocytes are sensitive to cold.

Silicon Gel Sheeting

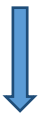
Topical silicon gel sheeting has been proved to relieve the most notorious symptom of keloids which is pruritus in a number of studies and has also showed significant softening of keloids and hypertrophic scars. These dressings are kept over the keloids in contact and then secured with a tape for about 12 hours each day.

It acts by the modification of the fibroblasts caused due to the hydration provided by the dressing.[57]

Hydration



Fibroblast modification



Softening of keloid

These are used as preventive strategy post-surgeries, after the healing of the operative wound. These are the most accepted treatment option among all the non-invasive therapies as there are no significant side effects.

Intralesional Corticosteroids

It has been the most accepted therapy and considered the first line of treatment for hypertrophic scars and keloids. Several studies have reported the softening of the texture, flattening of the lesion as well as improvement in the symptoms such a pruritus and tightening after being given intralesional triamcinolone acetonide.[58,59,60]

It has been observed that the rate of recurrence is significantly decreased when this is used in combination with surgical excision.

The most efficiently and commonly used intralesional corticosteroid, triamcinolone acetonide is either given individually or in combination with a local anaesthetic. When diluted with lidocaine, it has been proved to reduce the pain and discomfort caused due to the injection. Corticosteroids act by inhibiting the synthesis of collagen and also have an anti-inflammatory effect.

Decreased gene expression

Collagen synthesis suppression

Keloid regression

Although this treatment option is very effective and widely accepted, several adverse effects that make it a ‘not very desired procedure’ include:

1. Pain during the injection
2. Atrophy
3. Telengiectasia
4. Hypopigmentation
5. Depigmentation

Also, it is sometimes very difficult to push the drug into the fibrous mass which can be made easy by either injecting hyaluronidase or delivering cryotherapy or by using pulsed dye laser.

Interferon Alpha

Interferon alpha-2b was first used by Duncan and Berman[61] as it had a anti-proliferative effect. They treated a keloid with 2 doses of interferon alpha-2b 1.5 million International Units, with an interval of 4 days and have found that the keloid reduced to half the original size.

IFN acts by reducing the synthesis of type 3 and type 1 collagen from the fibroblasts, thus proving its anti proliferative property.

5-Fluorouracil

5-Fluorouracil is a pyrimidine analogue with a anti-metabolite activity. Fitzpatrick demonstrated the efficacy of this drug with and without steroid in the treatment of scars and keloids.[62] It acts by inhibiting the proliferation of fibroblasts. Initially, the injections need to be given frequently, about 1 to 3 per week, followed by longer intervals of four to six weeks between 2 doses.

Injections need to be given only in the hard or indurated area till a mild blanching is noticed. As these are very painful, they can be given in combination with triamcinolone acetonide or after administration of a local

anaesthetic. Combining a corticosteroid reduces the inflammation in addition to relieving the pain.

5-FU

Inhibition of fibroblast proliferation

Decreases scarring

Intralesional Bleomycin

Bleomycin has shown to flatten keloids when given at a dose of 1.5 International Units/ml intralesionally.[63]

Other agents used for the treatment of keloids

a) Imiquimod (Local immunomodulatory)

b) Intralesional Verapamil

   It is said that it acts by the inhibition of endothelial growth factor and interleukin - 6

c) Calcium antagonists

d) Tamoxifen

e) Tretinoin

f) Tacrolimus

g) Tranilast

h) Zinc

i) Vitamin E

j) Pentoxifylline

k) Colchicine

l) Calcium antagonists

LASERs for Keloids

Apfelberg et al.[64], first reported the use of lasers on keloids by irradiation with carbondioxide and argon lasers in 13 patients out of whom, only 1 patient showed improvement.

Later after multiple experiments with argon and CO_2, Nd:YAG and PDL were evaluated for the treatment of keloids, with variable outcomes. However, pulsed dye laser, later showed promising results. Several authors suggested that other therapeutic modalities like intralesional steroids, silicone gel sheets or pressure therapy should be advocated in combination with lasers.[19]

Pulsed dye laser was reported to significantly control pruritus in both keloids and hypertrophic scars.

Possible mechanism by which LASERs control pruritus:

Modification in

a) Neuropeptide Y
b) Vasoactive intestinal peptide
c) Substance P
d) CGRP (calcitonin gene-related peptide)

Allison et al.[65] reported a diminished number of mast cells as another mechanism of pruritus reduction which was however, contradictory to the theory put forth by Alster and Williams[66] who suggested a rise in the number of mast cells after treatment with laser.

Lasers have been reported to show an improvement in the texture of the skin, the cause of which is suggested be the result of remodelling of collagen.

Also, improvement in the pain, tightness and erythema has been reported after Laser therapy.

Adverse effects of Laser treatment

1. Post-operative purpura:

   It is a common adverse effect of PDL and may persist for upto 1 week after the procedure.

2. Hyperpigmentation:

   This may be due to the heat injury of the epidermis resulting from the absorption of melanin. It is more frequent in dark skinned patients.

3. Transient hyperpigmentation / hypopigmentation
4. Blistering

Mechanisms of action

The exact mechanism by which Lasers cause scar regression is not known, however, the available theories are based on the phenomenon of ‘selective photothermolysis’.

Light energy from a vascular laser (PDL)

Absorbed by haemoglobin, heat generation

Coagulation necrosis

Hypoperfusion & hypoxia, resulting in neocollagenesis

Heating of collagen fiber + Breakage of disulphide bonds

Realignment of collagen fibres

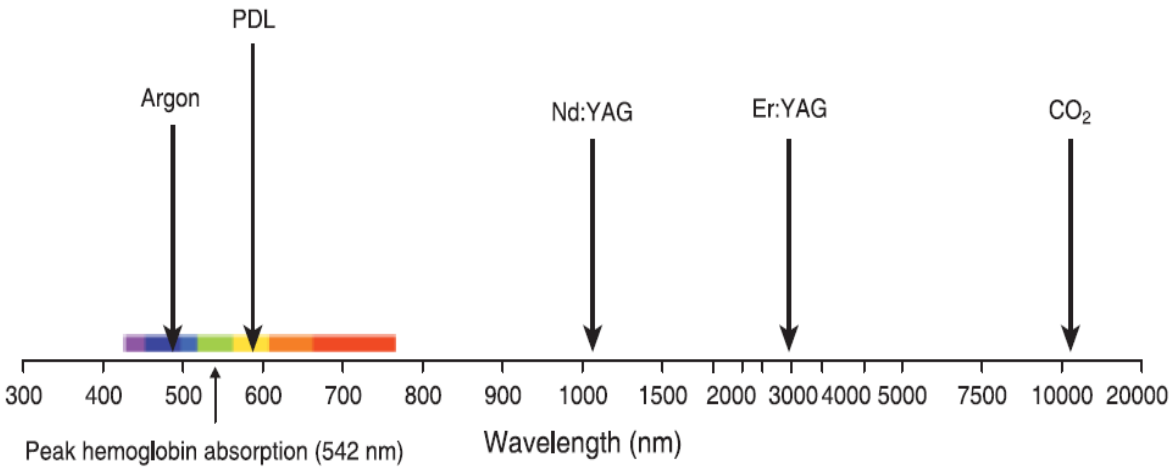
Decreased fibroblast proliferation and reduced deposition of type 3 collagen

Another possible mechanism is the apoptosis induction along with ERK (extracellular signal-regulated kinase) upregulation and activity of p38 MAP (mitogen-activated protein) kinase.As it is quite evident that the transforming growth factor – beta 1 (TGF- β1) is associated with

modulation and induction of collagen formation, PDL causes regression of keloids by downregulating TGF-β1.

Nowak et al.[67] reported that the superpulsed carbondioxide laser causes the release of bFGF (basic fibroblast growth factor) and inhibition of TGF-β1 release in keloids.

Paquet et al.[68] have shown that induction of anoxia by capillary destruction using lasers alters synthesis of collagen by fibroblasts and degradation mediated by the release of metalloproteinase.



Wavelengths of LASERs used for hypertrophic scars and keloids[69]

Factors influencing the outcome of LASER treatment

a) Wavelength
b) Fluence
c) Spot size
d) Scar location
e) Scar duration
f) Combination therapy

Wavelength

Selection of the precise wavelength is the most crucial factor that determines the outcome of the treatment. Several lasers with different wavelengthsstarting from 488 to 1064nm have been analysed for the treatment of keloids and hypertrophic scars. The pulsed dye laser that has a wavelength of 585 nm has been demonstrated to be the most efficacious laser influencing the scar growth, although, there are very few studies proving the same.

It is hypothesised that the mechanism of action by which lasers cause regression of keloidal scars is by targeting the haemoglobin, and therefore the lasers with a wavelength closer to the absorption peak of oxyhaemoglobin (542 nm) are thought to be the most successful.

These kind of lasers do not have a deeper penetration and are hence are not useful for deeper vessels.

Carbondioxide laser which has a wavelength of 10,600nm has not given satisfactory results in the scar ablation, with 39-92% rates of recurrence.[71-75]

The Nd:YAG laser which has a wavelength of 1064nm has proved to specifically supress the production of collagen in the cultures of fiboblasts.[76]

A recent study on frequency doubled Nd:YAG (532 nm) laser demonstrated significant success when compared to 585 nm – PDL in the treatment of 'pigmented hypertrophic scars'. This wavelength of Nd:YAG laser is very close to the oxyhaemoglobin absorption peak which is 542 nm, making this a better option for the treatment of keloids and hypertrophic scars.

The Erb:YAG laser with a wavelength of 2940 nm has been tried in adjunction with the carbondioxide laser by Cheng et al. in invitro studies in which they noticed a rise in the basic fibroblast growth factor (bFGF)

and a fall in the levels of transforming growth factor – beta 1 (TGF-β1) in the cultured fibroblasts of keloids.

The argon laser with a wavelength of 488 nm causes shrinkage of the collagen by localised thermal induction, thereby helping in scar regression and is known to give rise to a higher rate of recurrence ranging from 45-93 %.[73,78,79]

Carbondioxide laser in combination either with Er:YAG or pulsed dye laser proved to show satisfactory results when compared to monotherapy with either of the lasers.

Fluence

It is also very important to choose the appropriate fluence as a pulsed dye laser in low fluence has shown to cause an increase in the rate of production of procollagen and the same laser in high fluence was thought to cause focal thermal coagulation leading to a greater magnitude of adverse effects.[80,81]

The optimal fluence that should be used for the treatment of scars ranges from 3.5 J/cm^2 – 7.5 J/cm^2.[82-86]

A few investigators have suggested that a laser at higher fluence results in a better scar response, studies have not proved any significant dissimilarities in the outcome of the treatment.

Spot Size

It is another factor that needs to be kept in mind before starting the procedure as it plays an important role in the treatment outcome.

It is suggested to select a smaller spot size for a laser with higher fluence and a larger spot size for patients with dark skin to intensify the degree of penetration and to achieve better results.

Scar location

Although there are no globally accepted theories regarding the relation between the location of the scar and the efficacy of the treatment, Nouri et al. observed that scars on the face, shoulders and arms showed a good response when compared to those on the chest.[87]

Dierickx et al. also demonstrated a good outcome for scars over the face.[83]

But, Alster and Nanni[88] proposed no association between location of the scar and treatment outcome.

Scar Duration

It is thought that age of a scars plays a role in the response to treatment as differently aged scars respond in different ways to the same therapy.

Some researchers have showed that scars which are less than 1 year old can be treated easily when compared to older scars, but the other set of authors disagree with the same.

Combination Therapy

Adjunctive use of intralesional corticosteroids, 5-fluorouracil and/or hydroquinone has been proved to be very helpful in the treatment of keloids.[89]

Combination therapy of PDL with intralesional triamcinolone acetonide (10-20mg) brought about a very significant improvement of pruritus, although, there was no sufficient regression of scar. It was reported that higher conc. Of steroids would have given a better outcome, although, with a higher risk of adverse events like telangiectasia and atrophy of skin.

Hydroquinones proved to be efficacious in enhancing the desired result by decreasing the epidermal pigmentation.[82]

Even after 3 decades of progress in the field of laser therapy for keloids and hypertrophic scars, treatment of keloids still remains a therapeutic challenge. However, combination therapy with lasers such as PDL, Nd:YAG and carbondioxide lasers have shown promising results.

PULSED DYE LASER

Although the first laser used for the treatment of keloids and hypertrophic scars was argon laser of the continuous wave type, the publication of the phenomenon of 'selective photothermolysis' by Anderson and Parrish[12] in the 1980s created a revolution in the history of laser technology. The application of this concept of 'selective photothermolysis' allowed specific and controlled destruction of a target with a very little damage to the surrounding normal tissue.

This theory brought about the invention of pulsed lasers which were highly target – specific and selective, thereby decreasing the amount of damage occurring to the surrounding unwanted tissue.

In the late 80s, the pulsed dye laser with a wavelength of 585 nm that is vascular specific (oxyhaemoglobin is the chromophore) became very popular and its effectiveness in the treatment of vascular lesions was known widely.

Later, a few clinicians started deploying the pulsed dye laser for reducing the persistent erythema associated with keloids and hypertrophic scars. In 1933, Alster et al. showed that PDL treatment improved the

texture of the skin, pliability and bulk of argon laser-induced scars. Subsequently, several studies demonstrated a very significant improvement of surgical as well as traumatic hypertrophic scars, with a couple of sittings with the PDL.[83,84,91,92]In the early 1990s, it became quite evident that PDL could emerge as the most effective treatment with long standing improvement of various erythematous keloids and hypertrophic scars without any recurrence.

It was also proved that the keloid sternotomy scars and burn scars also respond to the laser therapy with PDL without any recurrence.[66,88]

Construction of a PDL

Pulsed dye laser, powered by a flash-lamp, emits a beam of yellow light which is pulsed and has a wavelength of 585 nm.[93] The lasing or the active medium is a fluorescent organic dye that is dissolved in a liquid and contained in a transparent cell.[94] The chemical structure of the dye, the type of solvent, and other additives used determine the lifetime of the device, which may be affected, because of the decomposition of the dye or the solvent, when exposed to heat.[94] The dye that is efficacious and has a longer life time is Rhodamine 6G. The transparent chamber housing the dye is surrounded by a flash lamp that is able to produce pulse duration of 450 μs.[94]

The pulse duration of 450 μs is at the lower side of the range of the thermal relaxation time of the vasculature of the skin[95] which ranges from 200−3000 μs for vessels with a diameter of 10−40 μm.[94] At a wavelength of 585 nm and a pulse duration of 450 μs, PDL penetrates to about 0.2 mm belowthe epidermis, which can also be increased by increasing the wavelength.[94]

Usage of the first-generation PDLs with smaller spot size, shorter pulse durations and wavelengths along with high fluence resulted in very high rates of complications, of which, the most common was the cosmetically unacceptable post treatment purpura that appears immediately and lasted for a week or two.[96]

Adverse effects of PDL

a) Post-operative purpura,
b) Hyper pigmentation or hypopigmentation,
c) Hypertrophic scarring,
d) Atrophy

The pulsed dye lasers that have been developed more recently, have larger spot sizes and longer wavelengths of 590 nm, 595 nm and 600 nm, thereby, being able to penetrate to a greater depth. They also have longer pulse durations corresponding to the size of the feeding vessel, and provide epidermal cooling to ensure a safe delivery of higher fluences without complications. Fluences ranging from 3 to 10 J/cm^2 are deliveredwith spot sizes of 2, 3, 5, 7, or 10 mm and an elliptical spot size of 2 × 7 mm.

Immediate purpura following PDL with high peak energy and short pulse duration is thought to be produced due to a photoacoustic rupture of capillary walls resulting in leakage of RBCs into the extravascular tissue.[96]

PDL with a longer pulse duration attains a lower peak energy, resulting in slower application of thermal energy to the blood vessels. This in turn, removes or decreases the photoacoustic effect to the capillary vessel walls with minimal leakage of red blood cells thereby, resulting in ‘no purpura’ while maintaining the clinical efficacy.[96]

PDL can safely be employed in infants and children.[97] It has been suggested that multiple and recurrent sittings with PDL should be preferred in order to prevent complications in children.[97] This was initially used for port wine stains, but is now being used extensively for a variety of acquired

vascular lesions like pyogenic granulomas, telangiectasias, cherry angiomas, venous lake and poikiloderma of civatte.

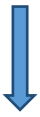
However, the maintenance and servicing of the pulsed dye laser is very difficult as the dye and the flash lamp have to be verified at the end of each year and need to be replaced if required. The laser machine has to be turned on at least once in a day to facilitate the circulation of the dye within the machine for a proper functioning.

Mechanism of Action

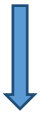
The exact mechanism by which PDL causes regression of keloids and hypertrophic scars is not known, however, the plausible theories are based on the phenomenon of 'selective photothermolysis'. Most accepted hypothesis is as follows:[70]

Light energy from a vascular laser (PDL)

Absorbed by haemoglobin

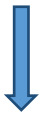


Heat generation

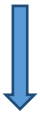


Coagulation necrosis

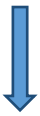
Decreased microvascular perfusion &tissue hypoxia



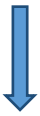
Neocollagenesis



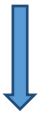
Heating of collagen fibers



Dissociation of disulphide bonds



Realignment of collagen fibres



Decreased fibroblast proliferation and reduced deposition of type 3 collagen

Other authors suggest a role of mast cell factors such as histamine, interleukins etc. in modifying the metabolism of collagen.[66]

Technique

The procedure of PDL therapy is done on an out-patient basis without the need of an admission in the hospital or general/spinal anaesthesia as there is very minimal discomfort/burning sensation. However, if desired, local anaesthesia can be delivered using a topical lidocaine/prilocaine cream under occlusion for 30 to 45 minutes.

Skin should be wiped with a wet gauze prior to the irradiation with laser in order to remove dirt or any makeup. If the procedure has to be performed over sensitive areas of the body such as fingertips, breast, perineum etc, intralesional anaesthetic injections or nerve blocks may be of help. Protective eye wear should be worn by the patient, doctor and also the others assisting the procedure in the room.

Technically, a series of adjacent, non-overlapping pulses of laser should be delivered across the entire area of the scar at each session. The procedure needs to be done with a perfectly chosen energy density depending on the thickness, location and duration of the scar, keeping in mind the patient’s skin type.

If the first treatment session results in a good response, the energy density should be kept constant on subsequent sessions. If the response is minimal, the fluence has to be increased by 10%. However, if there is post-operative blistering or crusting, a lower energy density should be chosen for the next visit.

After the procedure, the patient needs to be counselled about avoiding unnecessary manipulation of the lased areas. He/she should also be advised application of sunscreen, avoidance of sun exposure and use of topical antibiotics if necessary. The treated area should be evaluated after a period of 6-8 weeks and next session of laser therapy done if required.

If the treated area develops hyperpigmentation, next treatment session should be postponed to avoid the interference of the therapy from a competing target (or chromophore), like melanin. Such cases can be treated with creams containing hydroquinone to accelerate the fading of pigmentation.

Hypertrophic scars regress by about 50-80% after a couple of treatment sessions. However, keloids or very fibrotic hypertrophic scars require more number of sessions with other therapies in combination for a good response.

FRACTIONAL CARBON-DIOXIDE LASER

The technology of fractional lasers is an emerging branch for the aesthetic improvement of scars, striae, wrinkles and resurfacing. Since the 1980s, the concept of 'fractional photothermolysis' has revolutionized the field of laser resurfacing of skin.

Fractional laser technology is rapidly evolving and becoming popular when compared to ablative and non-ablative technology because of its safety profile without major side effects, reduced down time and efficient clinical outcome.

The first CO_2 gas laser was developed by C. Kumar N. Patel from India in 1964 and was operated at Bell Laboratories. It was initially used in the industrial field, though, it made its way into the field of medicine by 1970.[98,99]

The carbondioxide laser produced infrared light with a wavelength of 10,600 nm and was primarily used for tissue vapourization (water being the chromophore/target) and destruction of a variety of cutaneous lesions.[11]

Laser treatment of keloids and hypertrophic scars started with carbondioxide, argon and Nd:YAG lasers in the mid 1990s.

Principle of Fractional Photothermolysis

This concept was introduced by Manstein et al.[100] in the year 2004 in a device that emitted light in a pixilated pattern to the skin, creating an array of MTZs i.e. microthermal zones and producing thermal injury to the skin in tiny microscopic columns.[100]This process causes thermal ablation of epidermal and dermal tissue in microscopic columns that are regularly spaced in arrays over a fraction of the surface of the skin, unlike the ablative resurfacing which causes a confluent patch of dermal or epidermal thermal injury

Fractional photothermolysis, therefore induces thermal alteration of skin in fractions or columns, without ablating the intervening areas that immediately repopulate the lased area. Different machines provide different depth, size and number of MTZs and in the same way, in a single machine, by using different settings such as number of stackings and fluence.

A variety of desired results can be obtained. In each treatment session, the formation of zones is limited to only about 15 to 25 percent of the lased area. Each zone is about 100 to 160 μ in diameter and about 300 to 700 μ in depth. The above mentioned measurements for each zone are obtained when the energy used is 8-12mJ/MTZ, which is the usual setting used when treating the face of the patient.

The most important advantage with this type of machine is that the MTZ's density can be adjusted according to the requirement of the patient, which is usually maintained at 2000 MTZs/$cm^2$for every treatment session. This density is obtained when only 20% of the surface of the skin is treated. Transepidermal elimination is a phenomena where the dermo-epidermal debris which forms the MENDs (microscopic epidermal necrotic debris) is automatically cleared.[101-103]

Following this, there is induction of repair and reepithelialisation conducted by the columns of intact skin surrounding the affected skin. There is accumulation of the epidermal stem cells and the fibroblast derived neocollagenogenesis in the tissue where there was thermal ablation, as in ablative laser resurfacing.

The advantage with the non-ablative laser is that there is no epidermal ablation, dermal content extrusion even though it works on the same principle of production of microthermal zones.

This minimal downtime in this procedure makes it superior to other treatment options. The downtime is reduced as the major portion of the skin which is present in between the treated zones remains non-ablated, aiding the quick regeneration of the treated skin. These fractions are operated upon in each session and after 2 to 6 sittings the therapy is ended. [104]

Advantages of fractional lasers:

a) Suitable for dark skin
b) Minimal downtime
c) Reduced postoperative edema and erythema
d) Reduced dyschromic changes

The results obtained in the ablative resurfacing are much better when compared to the non-ablative or to the fractional. But, due to the minimal downtime and quick healing, the latter have become the treatment of choice for most of the patients.

Ablative Laser Systems

The field of dermatology has evolved greatly in the last decade but the field of laser therapy had the most unprecedented progress. Since the last 25 years, the scanned and the pulsed $CO_2$and the erbium:YAG lasers were excessively used all over the world. The success of this laser therapy for the treatment of

- Atrophic scars
- Dyschromias
- Photo-induced facial rhytides
- Photo-damaged skin

is well known all over the world.

Continuous wave CO_2system was one of the first CO_2 lasers used to produce large amounts of heat deposition and would cause charring of skin due to the enormous delivery and absorption of energy.

The unforeseeable amount of thermal necrosis and the formation of scar resulting from these CW lasers interdict the application of this laser for facial resurfacing.

With the evolution of the scanned, pulsed and the high energy lasers, it has become extremely safe to use these lasers over delicate areas such as the face. It is now possible to use the laser to such a precision that it became the therapy of choice for the facial resurfacing. These lasers were able to produce a limited tissue ablation with minimal coagulative necrosis of the structures in the near vicinity. Due to its minimal side effects and ease of use, it became the gold standard for facial rejuvenation.

When the patients with photo-damaged or scarred skin were treated with this laser, it produced remarkable results in the clinical and histopathologic aspect. The remodelling and collagen shrinkage, which were most probably the reason for prolonged clinical improvement, required 1 or 2 additional passes in comparison to the epidermal ablation which occurred after a single pass of the CO_2laser therapy.

The dissociation of the interpeptide bonds occurs when the skin temperature increases to more than 62°C, as a result, there is a conformational change within the collagen structure that reduces the size of the moiety by almost two thirds of its normal length. The pathomechanism of prolonged neocollagenogenesis and remodelling after the therapy is not clear. However, it is assumed that this effect is generated due to the thermal desiccation along with the shrinking collagen. As there is exaggerated expression of actin post treatment, there is the scaffold

formation due to the shrinkage, which acts as a support for the deposition and remodelling of the wound. There is around 50% improvement in the atrophic scar depth, rhytide severity and skin tone in patients treated with CO_2 laser. But, because of its high postoperative morbidity, it is not usually considered.

LONG–PULSE Nd:YAG LASER

The operation of the first Nd:YAG Laser was demonstrated by Joseph E. Geusic et al.[105] in 1964 and its high emission was proposed to be useful in coagulating large blood vessels.[106]

Long pulse Nd:YAG has now become a point of interest in the treatment of keloids as it has shown to exert its effects on the microvasculature of the dermis, as well as, metalloproteinase.

Rationale for choosing Nd:YAG

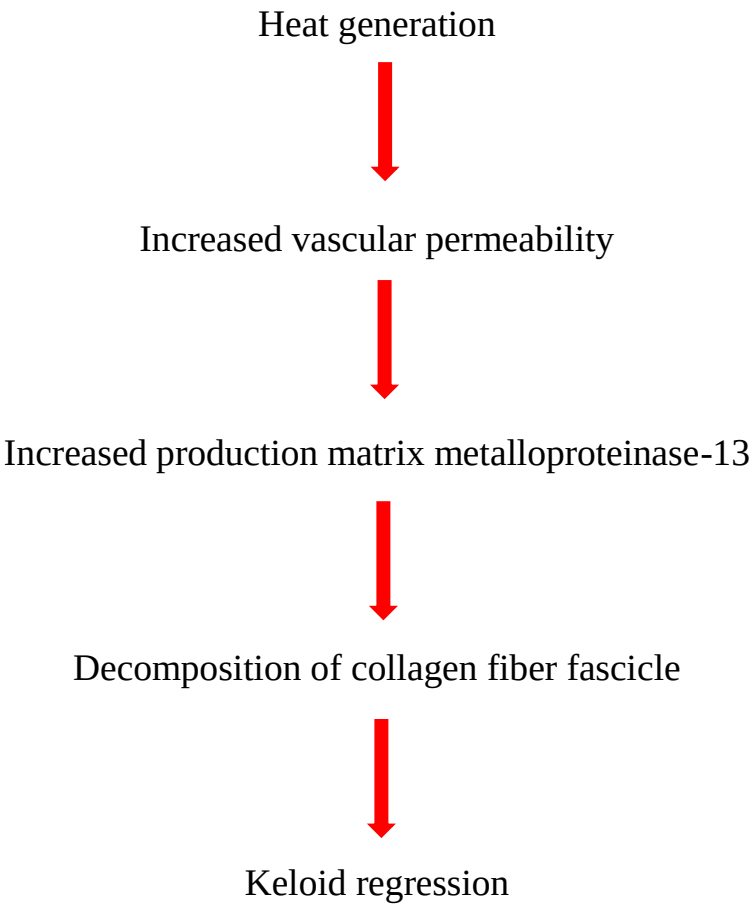
a) Dual mode of action: On both collagen and vessels.
b) Non-invasiveness
c) Good tolerability
d) Safety profile

Although PDL was found to be very effective in the treatment of cutaneous vascular diseases, it had a disadvantage of not being able to penetrate beyond the papillary dermis and this is where, long pulse Nd:YAG (neodymium-yttrium-aluminium-garnet) with a wavelength of 1064 nm came into light as it can penetrate till the reticular dermis.

Therefore, its application in deep vascular diseases such as keloids and hypertrophic scars has been increasing.

It has been thought that this laser acts by exerting a suppression of neovascularisation in these scars which have a characteristic overgrowth of blood vessels resulting in the presence of collagen and nerve fibers in the reticular dermis.

Mechanism of Action



Sherman et al.[107] reported an in vitro study in the year 1988, which demonstrated a reduction in the production of collagen induced by Nd:YAG laser. Also, a decrease in the erythema and induration was documented by them.

It is said that the limited depth of penetration caused by the yellow light emitted by the pulsed dye laser leads to resistance to further therapy with PDL due to the absorption and scattering in the epidermis and dermis (depth of about 1-2mm).[107,108]

Therefore, the deeper blood vessels can be specifically treated using Nd:YAG laser with a wavelength of 1064 nm. Owing to the deeper penetration and lower absorption by haemoglobin, the Nd:YAG laser (1064 nm) has become a better option in the treatment of deep vascular lesions than the conventionally used shorter wavelength sources.

Pathological scar development may also be influenced by interactions between mechanical pulling force, inflammation around the keloid, production of collagen and angiogenesis which suggests that 1064 nm Nd:YAG laser therapy may be efficacious in the treatment of pathological scars as it could minimise the vascularity of these scars.[109,110]

This decreased vascularity may reduce the levels of cytokines or growth factors in the tissue, which, in turn, promote deposition of collagen.

However, the laser might not be efficacious if the scars continue to be subjected to strong mechanical forces at and around the wound site.

Abergel et al. have demonstrated a photobiological effect on the metabolism of collagen using the Nd:YAG laser.[76]

It was observed that there was a selective inhibition of the production of collagen in the human dermal fibroblasts derived from tissue cultures after the delivery of only 1.1 x 10^3 J/cm^2 of Nd:YAG laser.

Cohen and Diegelmann suggested that Nd:YAG has a unique ability of selectively suppressing the collagen synthesis without affecting the replication of DNA and cell viability.[111]

Adverse effects

a) Post inflammatory hyperpigmentation

b) Post inflammatory hypopigmentation

c) Thermal injury/burns

d) Infection

MATERIALS AND METHODS

Study design

Hospital based observational study

Period of study

This study was conducted over a period of 2 years in patients with keloids after obtaining approval from the Institutional Human Ethics Committee.

Research subjects

The study was carried out on patients attending the out-patient department of Dermatology, Venereology and Leprosy, PSG IMSR, Coimbatore.

Sample size

15 patients

Inclusion criteria

15 patients above 15 years of age with keloids were included in the study.

Exclusion criteria

1. Patients below 15 years of age,
2. Pregnant and lactating females, and
3. Patients with history of isotretinoin use in the last 6 months.

Consent

Written and informed consent (both in English and local language) was obtained from each patient prior to starting the treatment and taking photographs.

Photographic documentation

Photographs of every keloid included in the study were taken at baseline and before each session of LASER, using a DSLR Nikon D5100.

Procedure

After taking photographs, patients were locally anaesthetised by topical application of a eutectic mixture of lidocaine and prilocaine, over the keloid to be treated, under occlusion for 1 hour. The cream was then, wiped off with a wet gauge and the area to be lased was cooled with icepacks in order to prevent unnecessary thermal injury and discomfort.

Protective eye wear was provided to the patient, treating doctor and all the personnel assisting the procedure.

(Picture 1& 2)

Fractional CO_2 laser (Lumenis) was delivered. After 5 mins of cooling of the lesion, long pulsed Nd:YAG & pulsed dye laser using the Cynosure Cynergy Multiplex was delivered.

(Pictures 3 & 4)

Laser parameters

Fractional CO_2 LASER was delivered as follows:

Initial energy of 10mJ was delivered with increments of 5mJ at each sitting. Five minutes after the delivery of fractional CO_2, *Cynosure Cynergy multiplex* (long pulse Nd:YAG and pulsed dye LASER) was delivered at a PDL fluence of 4J/cm^2 in the 1st sitting with increments of 1J/cm^2 during each sitting, while keeping the fluence of YAG constant at 20J/cm^2.

Laser Sessions	PDL Fluence	Pulse width	YAG Fluence	Pulse width	CO_2 Energy
1	4 J/cm^2	2 ms	20 J/cm^2	15 ms	10 mJ
2	5 J/cm^2	2 ms	20 J/cm^2	15 ms	15 mJ
3	6 J/cm^2	2 ms	20 J/cm^2	15 ms	20 mJ
4	7 J/cm^2	2 ms	20 J/cm^2	15 ms	25 mJ
5	8 J/cm^2	2 ms	20 J/cm^2	15 ms	30 mJ

Post-procedure care

- The lased area was again cooled,
- Patient was counselled about adequate photo-protection and the utmost necessity of sunscreen application.
- Topical steroid was applied immediately post-procedure to reduce the inflammation

Laser Sessions

Every patient underwent 5 sittings of the treatment with fractional CO_2, PDL and long pulse Nd:YAG lasers at intervals of 4 weeks.

Adverse Events

a) All patients tolerated the procedure well.

b) No complications such as hypo or hyperpigmentation, ulceration, or infection were observed during the course of the study.

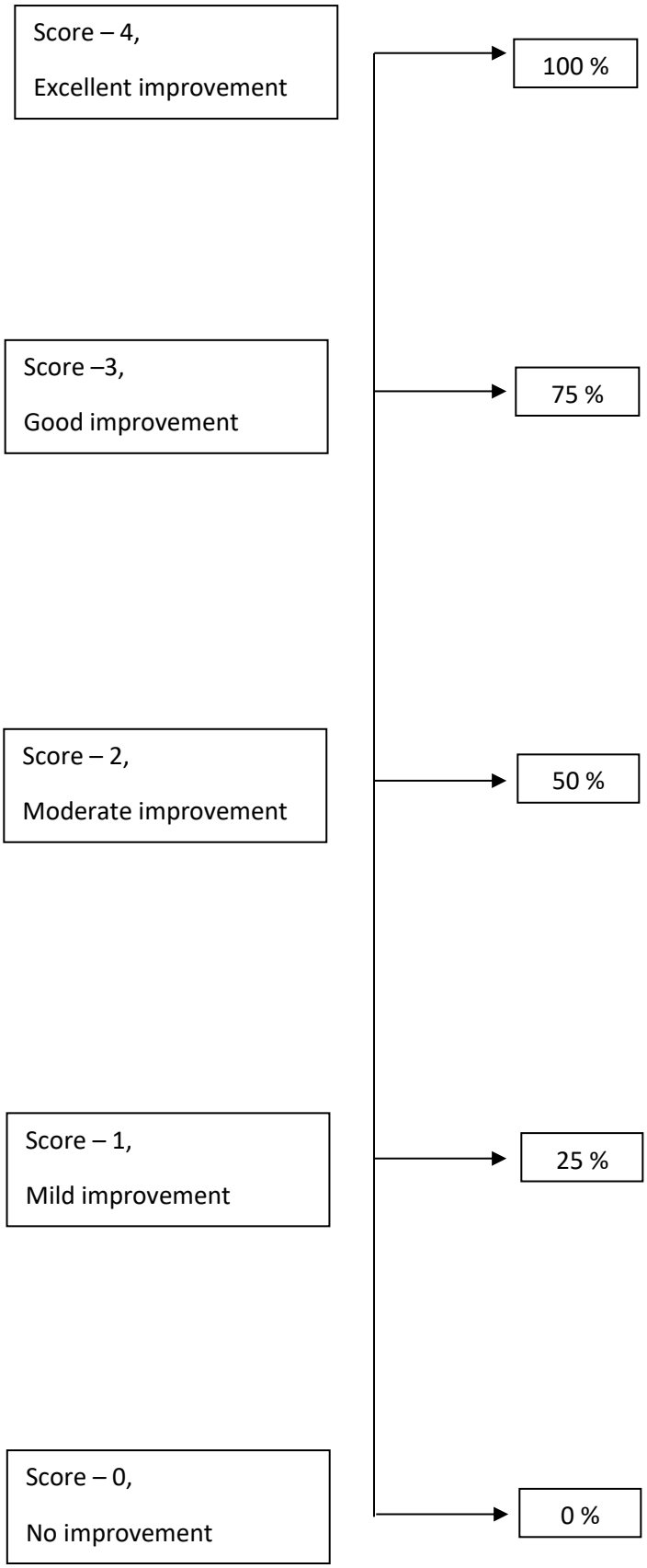
RESULTS

Out of the 15 patients included in the study, 11 patients completed the study. 4 patients discontinued the study and were lost for follow-up. Clinical improvement was monitored based on a VAS (visual analogue scale) graded by 3 blinded observers after assessing clinical photographs. The parameters analysed using the VAS were the colour, size and aesthetic impression.

A score of 0 for 'no improvement, 1 for 'mild improvement'(<25%), 2 for 'moderate improvement'(26%-50%), 3 for 'good improvement'(51%-75%) and 4 for 'excellent improvement'(76%-100%) was given.

0	**0%**	**No improvement**
1	**<25%**	**Mild improvement**
2	**26-50%**	**Moderate improvement**
3	**51-75%**	**Good improvement**
4	**76-100%**	**Excellent improvement**

VAS Scale used



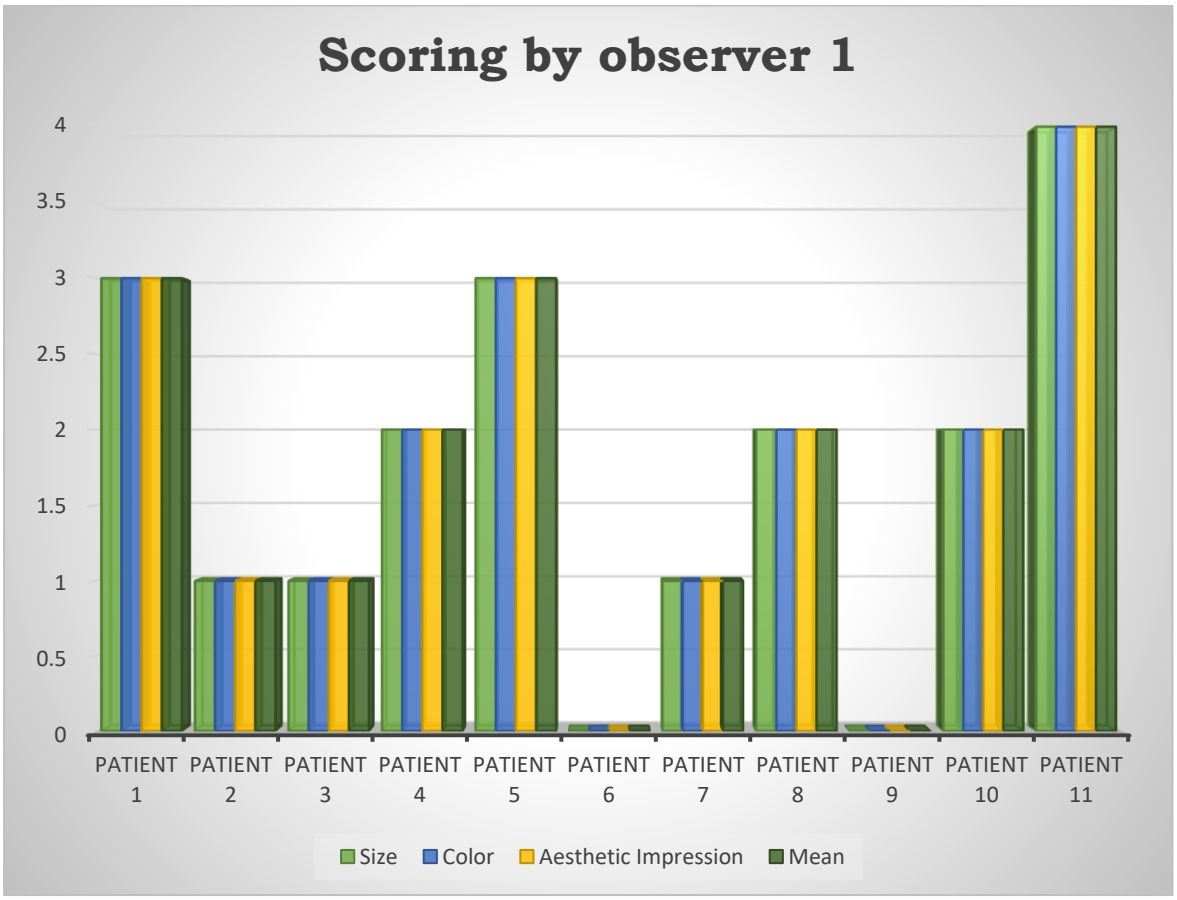
Analysis of the scoring

The mean of the scores given for all the 3 parameters (size, colour, aesthetic impression) by each observer for each patient was calculated and recorded.

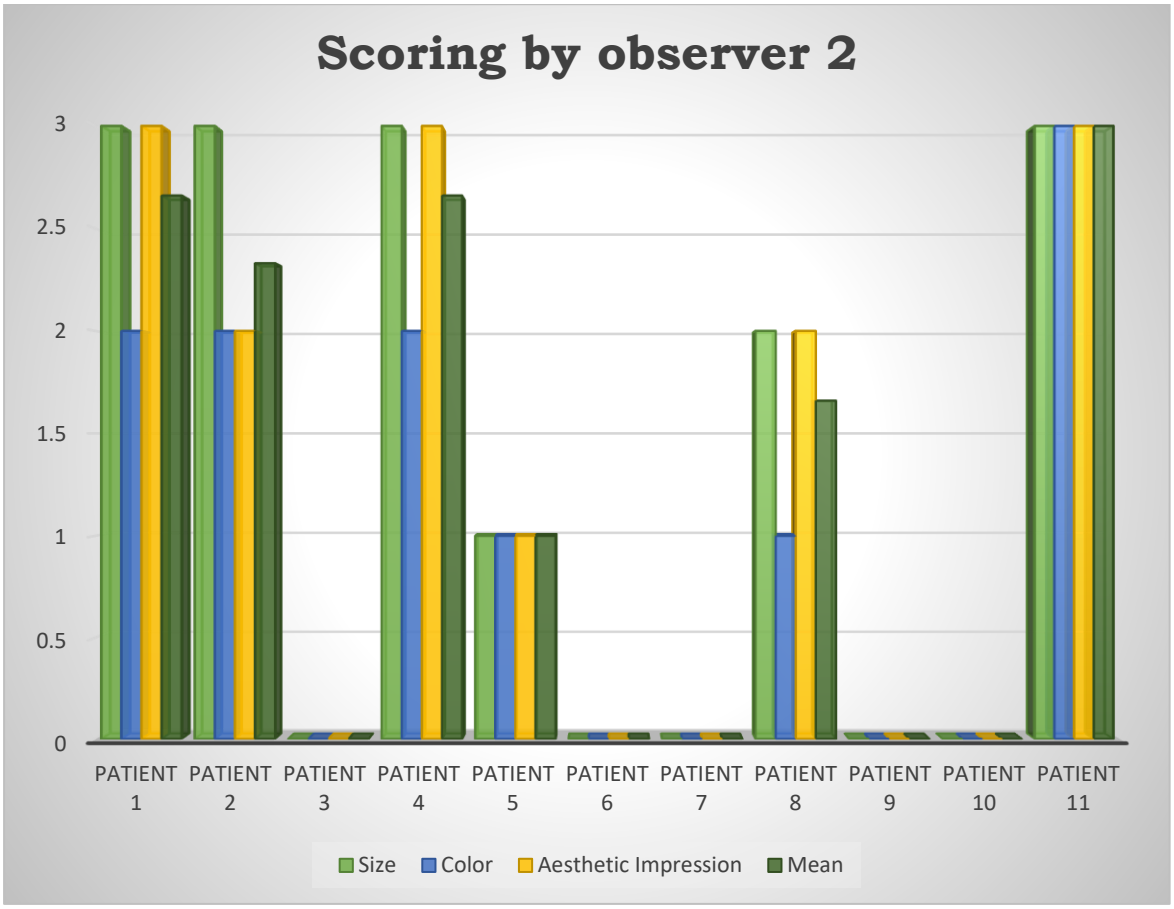
Then, the mean of the mean scores given by all the 3 observers was calculated and recorded as ‘overall improvement’.

Also, the mean of scores given for each parameter for each patient by all the 3 observers was calculated, aiding us to assess the improvement of individual parameters in each patient.

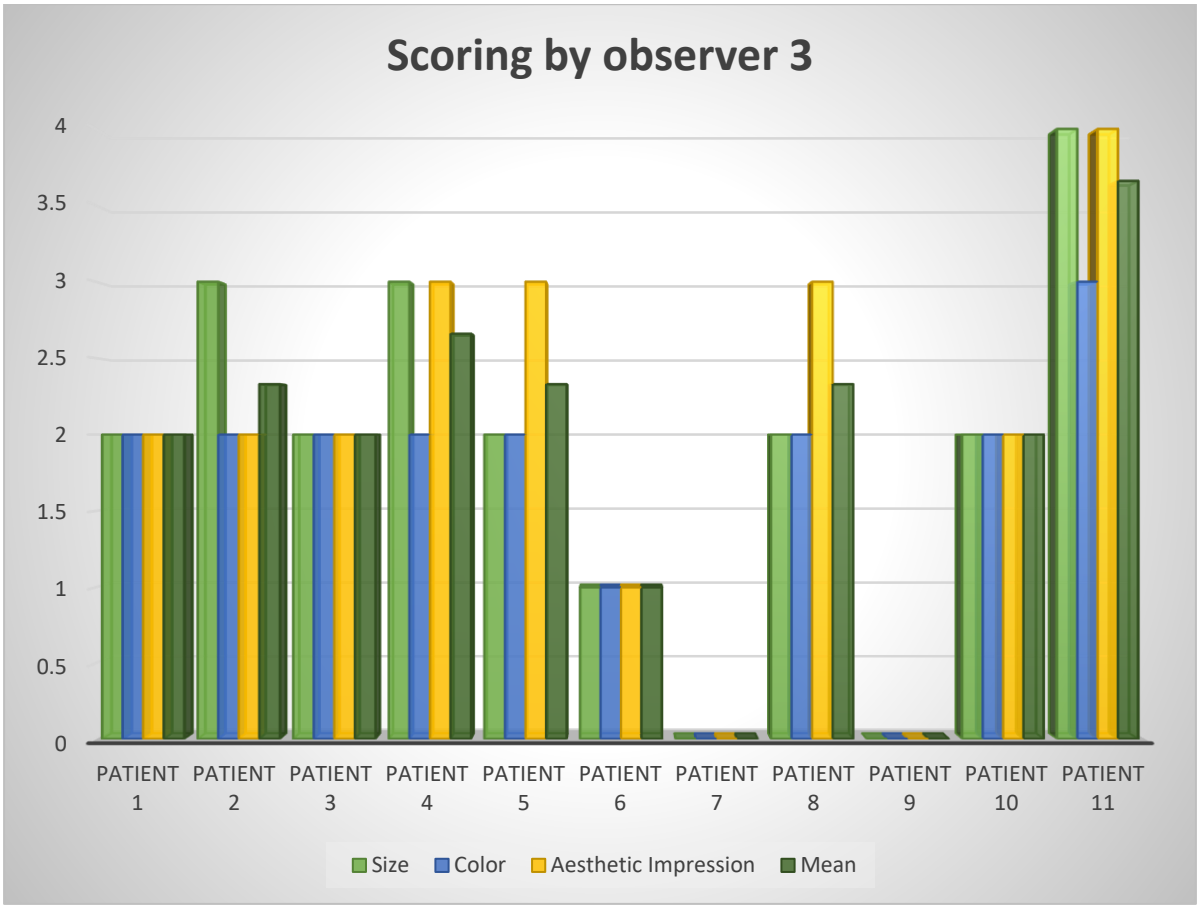
Scoring by observer 1				
S.No. of Patients	**Size**	**Color**	**Aesthetic impression**	**Mean score of 3 parameters**
1	**3**	**3**	**3**	**3**
2	**1**	**1**	**1**	**1**
3	**1**	**1**	**1**	**1**
4	**2**	**2**	**2**	**2**
5	**3**	**3**	**3**	**3**
6	**0**	**0**	**0**	**0**
7	**1**	**1**	**1**	**1**
8	**2**	**2**	**2**	**2**
9	**0**	**0**	**0**	**0**
10	**2**	**2**	**2**	**2**
11	**4**	**4**	**4**	**4**


Scoring by observer 1
4
3.5
3
2.5
2
1.5
1
0.5
0
PATIENT 1
PATIENT 2
PATIENT 3
PATIENT 4
PATIENT 5
PATIENT 6
PATIENT 7
PATIENT 8
PATIENT 9
PATIENT 10
PATIENT 11
Size
Color
Aesthetic Impression
Mean

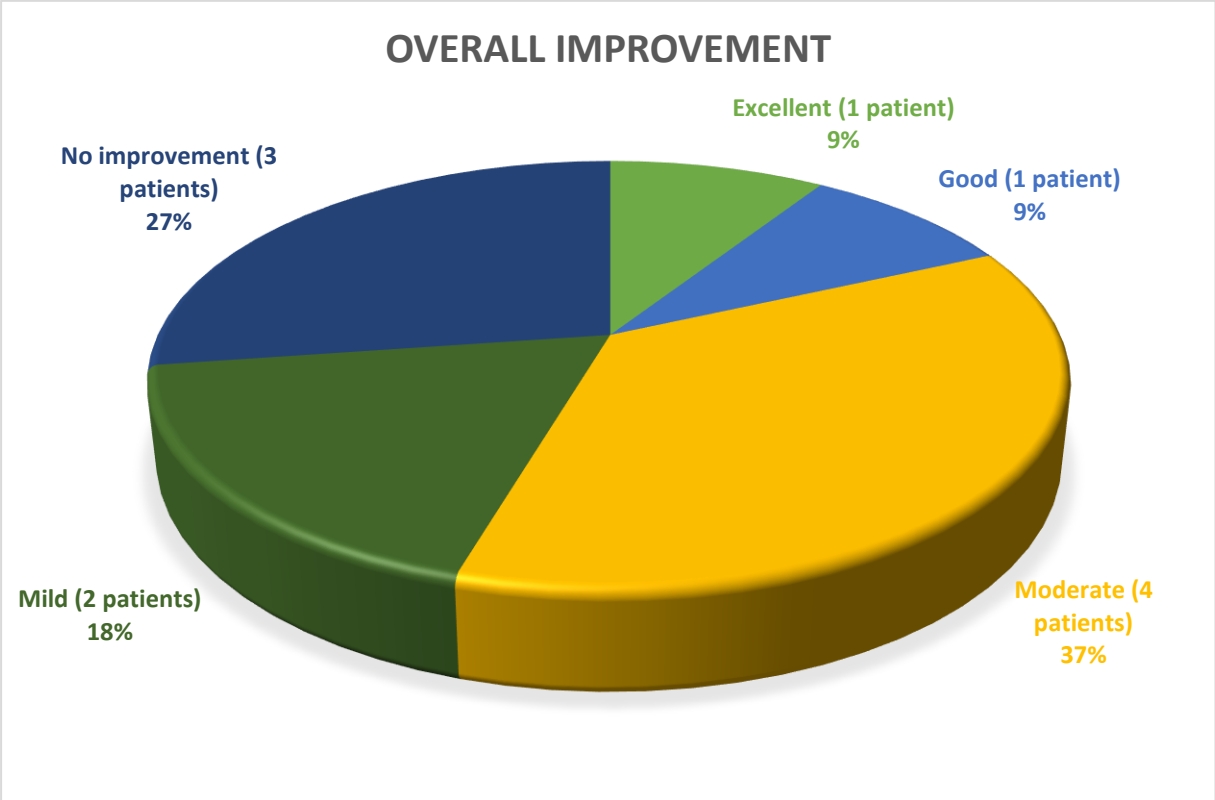
Scoring by observer 2				
S.No. of Patients	**Size**	**Color**	**Aesthetic impression**	**Mean score of 3 parameters**
1	**3**	**2**	**3**	**2.66**
2	**3**	**2**	**2**	**2.33**
3	**0**	**0**	**0**	**0**
4	**3**	**2**	**3**	**2.66**
5	**1**	**1**	**1**	**1**
6	**0**	**0**	**0**	**0**
7	**0**	**0**	**0**	**0**
8	**2**	**1**	**2**	**1.66**
9	**0**	**0**	**0**	**0**
10	**0**	**0**	**0**	**0**
11	**3**	**3**	**3**	**3**


Scoring by observer 2
3
2.5
2
1.5
1
0.5
0
PATIENT 1
PATIENT 2
PATIENT 3
PATIENT 4
PATIENT 5
PATIENT 6
PATIENT 7
PATIENT 8
PATIENT 9
PATIENT 10
PATIENT 11
Size
Color
Aesthetic Impression
Mean

Scoring by observer 3				
S.No. of Patients	**Size**	**Color**	**Aesthetic impression**	**Mean score of 3 parameters**
1	**2**	**2**	**2**	**2**
2	**3**	**2**	**2**	**2.33**
3	**2**	**2**	**2**	**2**
4	**3**	**2**	**3**	**2.66**
5	**2**	**2**	**3**	**2.33**
6	**1**	**1**	**1**	**1**
7	**0**	**0**	**0**	**0**
8	**2**	**2**	**3**	**2.33**
9	**0**	**0**	**0**	**0**
10	**2**	**2**	**2**	**2**
11	**4**	**3**	**4**	**3.66**


Scoring by observer 3
4
3.5
3
2.5
2
1.5
1
0.5
0
PATIENT 1
PATIENT 2
PATIENT 3
PATIENT 4
PATIENT 5
PATIENT 6
PATIENT 7
PATIENT 8
PATIENT 9
PATIENT 10
PATIENT 11
Size
Color
Aesthetic Impression
Mean

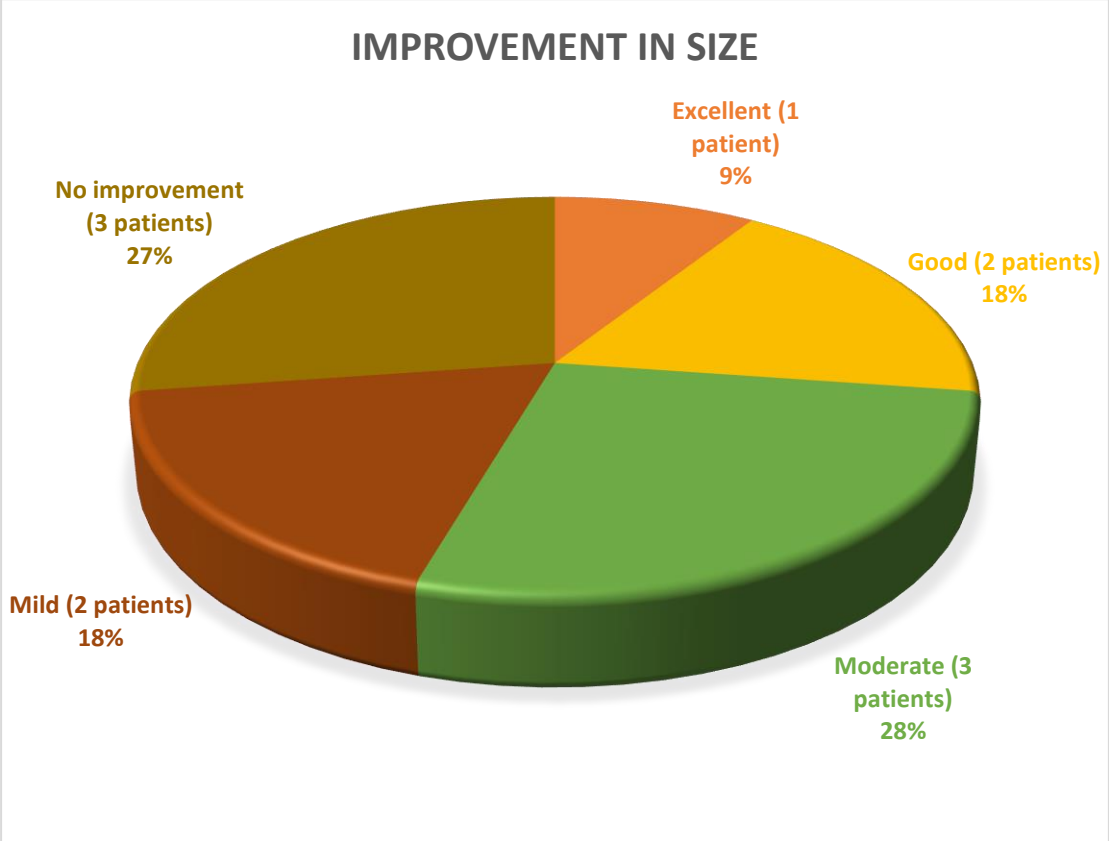
Mean of the scores given by 3 observers (‘Overall improvement’)						
S No. of Patients	**Mean score by observer 1**	**Mean score by observer 2**	**Mean score by observer 3**	**Mean of 3 mean scores**	**Rounded to**	**Interpretation of ‘overall improvement’**
1	**3**	**2.66**	**2**	**2.55**	**3**	**Good**
2	**1**	**2.33**	**2.33**	**1.88**	**2**	**Moderate**
3	**1**	**0**	**2**	**1**	**1**	**Mild**
4	**2**	**2.66**	**2.66**	**2.44**	**2**	**Moderate**
5	**3**	**1**	**2.33**	**2.11**	**2**	**Moderate**
6	**0**	**0**	**1**	**0.33**	**0**	**No**
7	**1**	**0**	**0**	**0.33**	**0**	**No**
8	**2**	**1.66**	**2.33**	**1.99**	**2**	**Moderate**
9	**0**	**0**	**0**	**0**	**0**	**No**
10	**2**	**0**	**2**	**1.33**	**1**	**Mild**
11	**4**	**3**	**3.66**	**3.55**	**4**	**Excellent**



Out of the 11 patients, 1 patient had excellent overall improvement (9%), 1 patient had good overall improvement (9%), 4 patients had moderate overall improvement (37%), 2 patients had mild overall improvement (18%) and 3 had no improvement (27%).

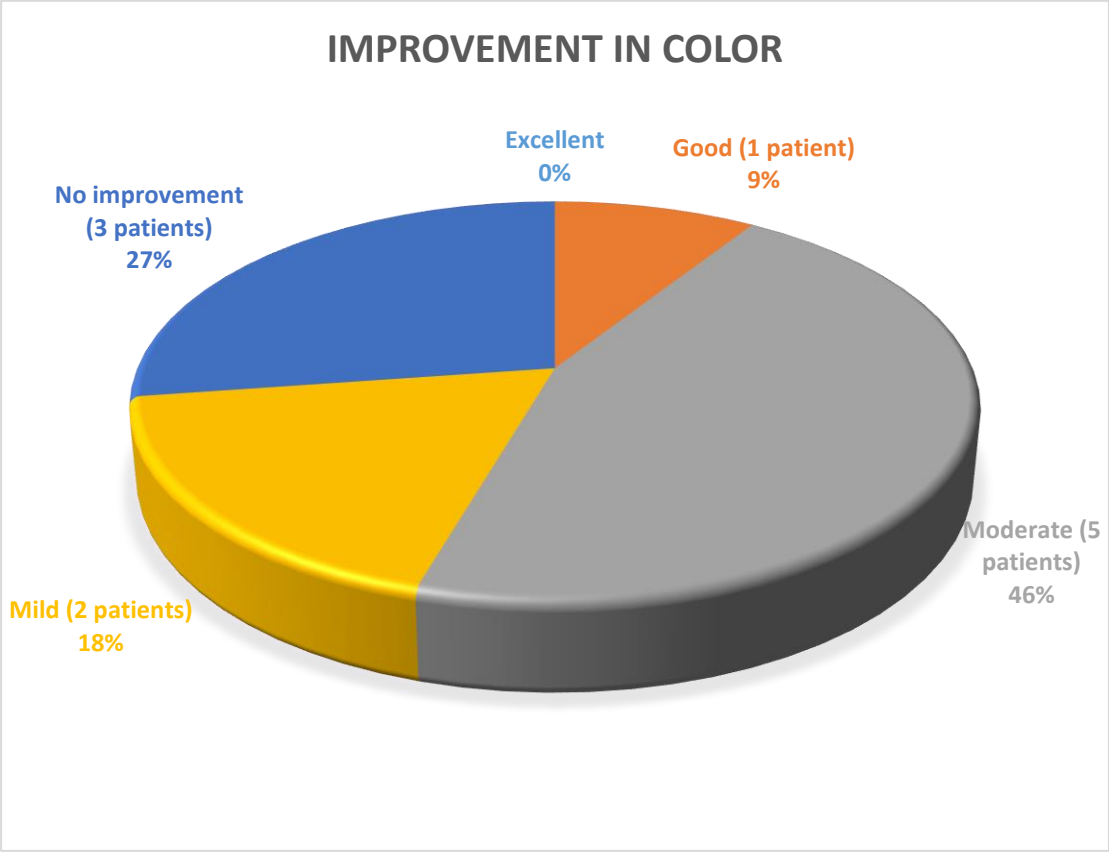
Mean of the scores given by 3 observers for 'Size'

S.No. of Patients	Score for 'size' by observer 1	Score for 'size' by observer 2	Score for 'size' by observer 3	Mean of 3 scores for 'size'	Rounded to	Interpretation of improvement in 'size'
1	3	3	2	2.66	3	Good
2	1	3	3	2.33	2	Moderate
3	1	0	2	1	1	Mild
4	2	3	3	2.66	3	Good
5	3	1	2	2	2	Moderate
6	0	0	1	0.33	0	No
7	1	0	0	0.33	0	No
8	2	2	2	2	2	Moderate
9	0	0	0	0	0	No
10	2	0	2	1.33	1	Mild
11	4	3	4	3.66	4	Excellent



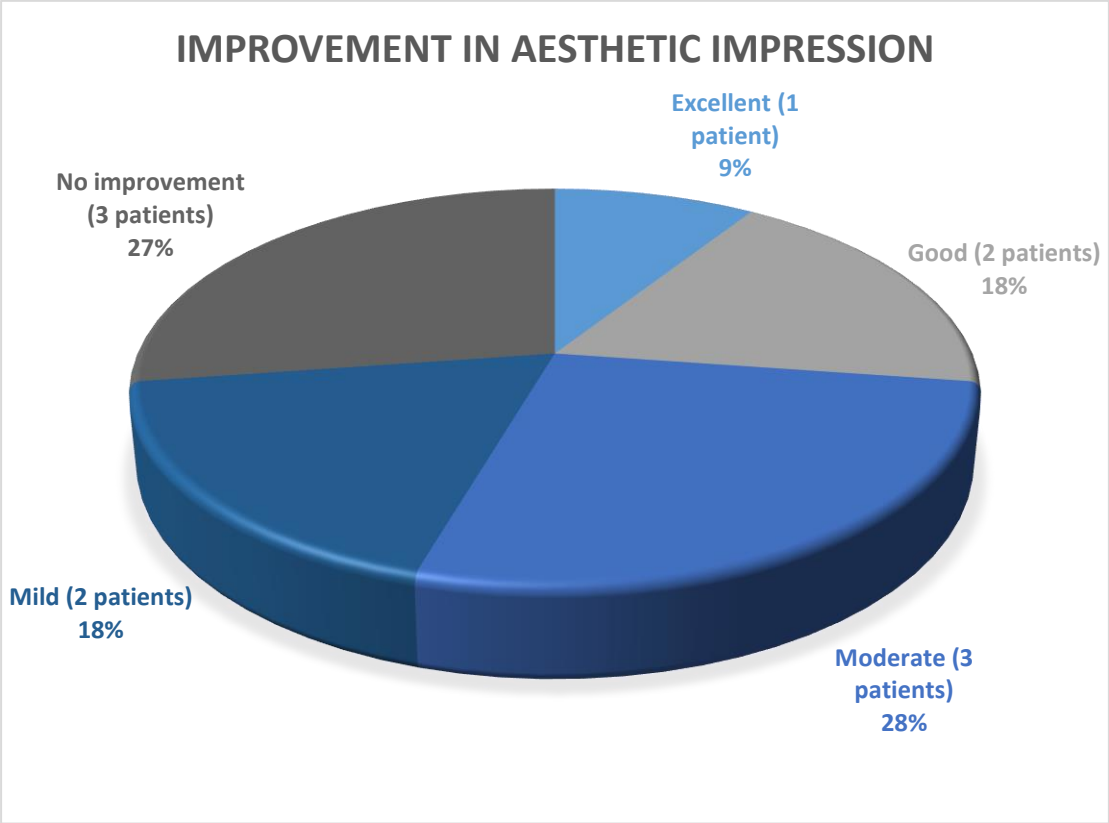
On analysing the size, we have observed that, out of the 11 patients, improvement in size was excellent in 1 patient (9%), good in 2 patients (18%), moderate in 3 patients (28%), mild in 2 patients (18%) and nil in 3 patients (27%).

Mean of the scores given by 3 observers for 'Color'						
S.No. of Patients	**Score for 'color' by observer 1**	**Score for 'color' by observer 2**	**Score for 'color' by observer 3**	**Mean of 3 scores for 'color'**	**Rounded to**	**Interpretation of improvement in 'color'**
1	**3**	**2**	**2**	**2.33**	**2**	**Moderate**
2	**1**	**2**	**2**	**1.66**	**2**	**Moderate**
3	**1**	**0**	**2**	**1**	**1**	**Mild**
4	**2**	**2**	**2**	**2**	**2**	**Moderate**
5	**3**	**1**	**2**	**2**	**2**	**Moderate**
6	**0**	**0**	**1**	**0.33**	**0**	**No**
7	**1**	**0**	**0**	**0.33**	**0**	**No**
8	**2**	**1**	**2**	**1.66**	**2**	**Moderate**
9	**0**	**0**	**0**	**0**	**0**	**No**
10	**2**	**0**	**2**	**1.33**	**1**	**Mild**
11	**4**	**3**	**3**	**3.33**	**3**	**Good**



Evaluation of the refinement in color revealed that no patient had excellent improvement, 1 patient had good improvement (9%), 5 patients had moderate improvement (46%), 2 patients had mild improvement (18%) and 3 patients had no improvement (27%).

Mean of scores given by 3 observers for 'Aesthetic impression'						
S.No. of Patients	**Score by observer 1**	**Score by observer 2**	**Score by observer 3**	**Mean of 3 scores for 'Aesthetic impression'**	**Rounded to**	**Interpretation of improvement in 'aesthetic impression'**
1	**3**	**3**	**2**	**2.66**	**3**	**Good**
2	**1**	**2**	**2**	**1.66**	**2**	**Moderate**
3	**1**	**0**	**2**	**1**	**1**	**Mild**
4	**2**	**3**	**3**	**2.66**	**3**	**Good**
5	**3**	**1**	**3**	**2.33**	**2**	**Moderate**
6	**0**	**0**	**1**	**0.33**	**0**	**No**
7	**1**	**0**	**0**	**0.33**	**0**	**No**
8	**2**	**2**	**3**	**2.33**	**2**	**Moderate**
9	**0**	**0**	**0**	**0**	**0**	**No**
10	**2**	**0**	**2**	**1.33**	**1**	**Mild**
11	**4**	**3**	**4**	**3.66**	**4**	**Excellent**



On the analysis of 'aesthetic impression', it was found that, out of the 11 patients, improvement in aesthetics was excellent in 1 patient (9%), good in 2 patients (18%), moderate in 3 patients (28%), mild in 2 patients (18%) and nil in 3 patients (27%).

DISCUSSION

Keloids are abnormal wound responses characterised by excessive deposition of collagen and glycoprotein. They occur in predisposed individuals as a result of connective tissue response to trauma or may sometimes, have a spontaneous development.

It has been investigated that the activity of fibroblasts, various growth factors, components of extracellular matrix, cytokines and immune response portray the cellular basis of exaggerated fibrogenesis leading to the formation of a keloid.

They are both aesthetically and symptomatically distressing for most of the patients. Keloidal scars may cause symptoms like pain, pruritus, restriction of movements and also aesthetic disfigurement leading to a significant amount of morbidity.

They give rise to both functional and aesthetic distress to the patient and have always been a therapeutic challenge, despite the innumerable modalities of treatment. Conventional methods for the treatment of keloids include surgical excision, dermabrasion, grafting, radiation, pressure therapy, silicone gel sheeting and intralesional corticosteroids.[2,3]

There has been a magnificent advancement in the field of lasers in dermatology over the last three decades. It is quite well-known that this revolution has changed the face of aesthetics and dermato-medicine.

Lasers have been in use for a variety of skin conditions like pigmented lesions, vascular anomalies, tattoos, hirsutism, keloids and hypertrophic scars, and also for skin resurfacing. They have become a well-known treatment modality for the management of keloids in recent years.

There are reports of keloid management with pulsed dye laser, fractional CO_2 and Nd:YAG laser individually and also in combination of CO_2 with PDL and CO_2 with Nd:YAG.

Here, we discuss a combination of all the 3 LASERs as therapy for keloids. A total of 15 patients above 15 years of age, with keloids were included in our study. If a patient presented with more than one keloid, only one keloid from each patient was considered for our study.

In a study conducted by Kono et al.,[112] 19 hypertrophic scars were irradiated with a pulsed dye laser which demonstrated that 16 lesions

(84%) showed significant flattening of the scar and reduction in erythema. Therefore, he suggested that treatment of scars with pulsed dye laser can help to reduce the erythema and size very effectively.

In our study, where we used a combination of 3 lasers, it was found that, out of 11 lesions, 8 lesions (73%) showed improvement in size and erythema/color which was almost in accordance with the study done by Kono et al.[112]

In 2004, Chan et al.[82] treated 36 scars in 27 patients with 585 nm – PDL and 66% reported clinical improvement which is slightly less than that of our study which showed improvement in 72% of patients.

Another study done by Nouri et al.[87] in 2003, revealed that all the 12 post operative scars treated with 3 sessions of pulsed dye laser (585nm) showed significant overall improvement and enhanced cosmetic appearance. However, in our study, improvement in aesthetic impression was seen only in 8 (73%) out of 11 patients.

Alster[4], in 2003, treated 22 hypertrophic scars using PDL and found that there was a very significant clinical and histologic enhancement. We

cannot compare our study with the above as we have not done a histologic evaluation.

Paquet et al.[68] treated 11 keloids with PDL in 2001 and proposed that there was no significant difference in the size and also in the refinement of erythema which is contradiction to the study done by us.

Connell and Harland[108] managed 10 recalcitrant keloids in 10 patients with a combination of PDL and intralesional steroid and reported that 7 of them had good improvement in the size and erythema. They have also stated that the results of this combined therapy are not only adjunctive, but also summative. In our study, we have not combined PDL with any other adjunctive therapy.

Shakespeare et al.[114] conducted a study with PDL on hypertrophic scars in 11 patients and showed that there was significant improvement in all the cases. In our study, there was significant improvement only in 8 out of 11 cases.

Alster and Nanni,[88] treated 40 hypertrophic scars with pulsed dye laser and found that all the lesions had reduction in the erythema with

improvement in the texture and pliability. In our study, reduction in erythema was seen only in 8 out of 11 patients.

In another study carried out by Dierickx et al.[83], 15 patients were treated with PDL and it was found that all the scars showed significant reduction in the scar erythema.

Goldman and Fitzpatrick[84] conducted a study using PDL on 48 patients with hypertrophic scars and demonstrated that all the scars resolved completely. However, in our study, only 1 out of 11 patients showed complete resolution.

A study done by Alster and Williams[66] in 1995 on keloid sternotomy scars using PDL showedsignificant improvement in erythema, scar size and pruritus, the results of which are in a close agreement with the study conducted by us.

Several studies using CO_2 laser were carried out to prove its efficacy in the treatment of keloids. Norris[72] in 1991, Apfelberg et al.[116] in 1989, Stern &Lucente[71] also in the year 1989 conducted studies to assess the efficacy of CO_2 laser on keloids but failed to prove promising results.

However, a study conducted by Alster et al.[115] in 20 patients with hypertrophic scars proved to be effective when the CO_2 laser was used in combination with PDL.

Kumar et al.[113] treated 17 keloids with Nd:YAG laser and demonstrated that 10 lesions (58.8%) completely resolved and 7 lesions (41.2%) showed only partial resolution. But our study demonstrated complete resolution only in 1 keloid (9.09%) and partial resolution in 7 keloids (63.64%).

Sherman & Rosenfeld,[107] in the year 1988 irradiated 17 keloids with Nd:YAG and found promising results with all the keloids. Similarly, Abergel et al.[117] conducted a study in 8 patients with keloids using Nd:YAG laser and demonstrated good results.

Several laser sessions are advised for obtaining promising response but lower fluences are suggested for the avoidance of adverse effects in patients with a higher melanin content in the skin.

CONCLUSION

LASERs are now being used to enhance keloids and hypertrophic scars. After their introduction for keloids and hypertrophic scars by Apfelberg et al.[64] and Castro et al.[118] in the 1980s, the field of lasers has been evolving magnificently and several lasers with varied wavelengths have been studied, although making it very difficult for a clinician to assess the efficacy of each.

Lasers used either singly or in combination may be effective to a varying degree in the treatment of keloids. We plan to conduct studies combining all other modes of treatments along with LASERs to determine if better results can be achieved.

Limitations

- The small sample size.
- The number of LASER sessions was limited to 5.
- The other modalities of treatment were avoided in order to assess the efficacy of LASERs alone.

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CLINICAL PHOTOGRAPHS

PROTECTIVE EYE WEAR FOR LUMENIS CO_2 LASER

PROTECTIVE EYE WEAR FOR
CYNOSURE CYNERGY MULTIPLEX

LUMENIS ACUPULSE CO_2 LASER

CYNOSURE CYNERGY MULTIPLEX LASER
(PDL + LONG PULSE Nd:YAG)

BEFORE STARTING THE TREATMENT

AFTER 5 SESSIONS OF LASER

BEFORE STARTING THE TREATMENT

AFTER 5 SESSIONS OF LASER

BEFORE STARTING THE TREATMENT

AFTER 5 SESSIONS OF LASER

BEFORE STARTING THE TREATMENT

AFTER 5 SESSIONS OF LASER

CONSENT FORMS

Informed Consent

We Dr. Ashwini Annabathula, Dr.Shanmuga Sekar and Dr. C.R.Srinivas of the PSG Institute of Medical Sciences and Research (PSG IMSR), are carrying out a study titled :

Fractional CO_2, Long Pulse Nd:YAG and Pulsed Dye Laser in the Management of Keloids.

Under the aegis of the Department Of Dermatology, PSG IMSR.

The objectives of this study are:

- To assess the efficacy of Fractional CO_2 , Long Pulse Nd:YAG and Pulsed Dye Laser in the management of Hypertrophic Scars and Keloids

The goal of this study is: To achieve reduction in the size of keloids and hypertrophic scars

Sample size: 15

Respondents are (population group & age group): 15 years and older men and women who present with Keloids to the Dermatology OPD

Location: Coimbatore, Tamil Nadu.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out (strike off items that are not applicable):

Initial interview (specify approximate duration): _____min.

Health education sessions:

No. of sessions: N/A

Approximate duration of each session: N/A min.

Clinical examination (specify details and purpose): The lesions to be treated will be examined prior to treatment.

Blood sample collection: N/A Specify purpose, discomfort likely to be felt and side effects, if any: Mild erythema and irritation

Final interview (specify approximate duration): N/A min.

If photograph is taken, purpose: To compare the before, after, and follow-up pictures of the treated lesions for assessment of improvement

Benefits from this study, if any: New modality of treatment in the management of Keloids.

If you are uncomfortable in answering any of our questions during the course of the interview/ blood sample collection, you have the right to withdraw from the interview / study at any time. You will NOT be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigators from the PSG IMS&R. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature/left thumb impression to indicate my consent and willingness to cooperate in this study.

Respondent ID: _____.

Signature / Left thumb impression of the Respondent

Signature of the Investigator

Signature of the witness

ஒப்பதல் படிவம்

தேதி

அஸ்வினி அன்னாபத்துல்லா ஆகிய நான் PSG மருத்துவக்கல்லூரியின் பிராக்ஷனல் CO_2 லேசர், லாங் பல்ஸ் என்.டி.யாக் லேசர் மற்றும் பி.டி.எல் லேசர் மூலம் வளர் வடுவினை அகற்றுதல் பற்றி ஆய்வுத்திறன் மேற் கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி : மரு.ச. சண்முகசேகர்

ஆய்வு மேற்கொள்வதற்கான அடிப்படை:

வளர்வடு தோலில் கட்டித் தழும்பை உருவாக்கும். அத்தழும்பை குணப்படுத்த பல்வேறு சிகிச்சை இருப்பினும் முழுமையாக குணப்படுத்தும் மருத்துவ முறைகள் மிகவும் குறைவு. இதன் மூலம் வளர்வடுக்கான புதியதொரு சிகிச்சை முறைபற்றி ஆய்வுத்திறன் மேற்கொள்கிறேன்.

ஆய்வின் நோக்கம் : வளர்வடுவை குணப்படுத்துதல்

ஆய்வு மேற்கொள்ளும் இடம் : **பி.எஸ்.ஜி மருத்துவமனை, தோல் பால்வினை மற்றும் தொழுநோய் துறை**

பரிசோதனைக்கு உட்படுத்தப்பட்டவர்களின் எண்ணிக்கை : 15

வயது வரம்பு : 15 வயதிற்கு மேல்

இடம் : தோல், பால்வினை மற்றும் தொழுநோய்துறை
பி.எஸ்.ஜி மருத்துவமனை, கோயம்புத்தூர்

இந்த ஆய்வுக்கு எங்களுக்கு ஒத்துழைப்பைத் தருமாறு தங்களைக் கேட்டுக் கொள்கிறோம். மேலும் இந்த ஆய்வுக்குத் தேவையான தகவல்கள் உங்களிடமிருந்து பெற்றுக் கொள்ளப்படும்.

உடல் பரிசோதனை : சோதனைக்கு உட்படுத்தப்படும் பகுதியை பரிசோதித்தல்

பரிசோதனையின் போது ஏற்படும் பக்கவிளைவுகள் தோல் : சிவந்துபோதல், எரிச்சல்

புகைப்படம் எடுக்கப்பட்டதா? அதன் நோக்கம் :

பரிசோதனைக்கு முன்பும், பின்பும் உள்ள மாற்றத்தை ஒப்பிட

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

ஆய்விலிருந்து விலகிக் கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும்

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியல் பங்கு கொள்ளவும் இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

PROFORMA

VARIABLES RECORDED

Name: Age: OP No.

Gender: Occupation:

Duration of the lesion/s:

History of previous treatment:

History of trauma/acne preceding the development of lesion:

Family History of Keloids: Y/N

MASTER CHART

MASTER CHART

S. No. of Patients	Scoring by observer 1				Scoring by observer 2				Scoring by observer 3				Overall Improvement (Mean of the mean scores by all the observers)	Overall Improvement (Score rounded to)	Interpretation of "Overall Improvement"
	Size	Color	Aesthetic Impression	Mean score of 3 parameters	Size	Color	Aesthetic Impression	Mean score of 3 parameters	Size	Color	Aesthetic Impression	Mean score of 3 parameters			
1	3	3	3	3	3	2	3	2.66	2	2	2	2	2.55	3	Good
2	1	1	1	1	3	2	2	2.33	3	2	2	2.33	1.88	2	Moderate
3	1	1	1	1	0	0	0	0	2	2	2	2	1	1	Mild
4	2	2	2	2	3	2	3	2.66	3	2	3	2.66	2.44	2	Moderate
5	3	3	3	3	1	1	1	1	2	2	3	2.33	2.11	2	Moderate
6	0	0	0	0	0	0	0	0	1	1	1	1	0.33	0	No
7	1	1	1	1	0	0	0	0	0	0	0	0	0.33	0	No
8	2	2	2	2	2	1	2	1.66	2	2	3	2.33	1.99	2	Moderate
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No
10	2	2	2	2	0	0	0	0	2	2	2	2	1.33	1	Mild
11	4	4	4	4	3	3	3	3	4	3	4	3.66	3.55	4	Excellent

ABBREVIATIONS

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LASER	Light Amplification by Stimulated Emission of Radiation
Nd:YAG	Neodymium-doped yttrium aluminium garnet
PDL	Pulsed dye LASER
CW	Continuous wave
Q-Switched	Quality switched
TGF β	Transforming Growth Factor β
IGF	Insulin-like Growth Factor
bFGF	Basic Fibroblast Growth Factor